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THE CHICAGO MEDICAL SCHOOL
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CHEMICAL CARCINOGENS — AN INTRODUCTION*

P. SHUBIK, B.M., B.Ch., D.Phil.**

The present symposium is, of necessity, restricted to a consideration of chemical carcinogenesis. So much fundamental biological research has been undertaken in the field of cancer research in the past 30 years that it has become virtually impossible to summarize all the facets of carcinogenesis in less than several volumes.

In order to maintain a perspective it must be constantly remembered that most neoplastic change occurs under totally unknown circumstances, although there are a series of *inducing factors* known experimentally. These include viruses, physical agents (ionizing radiations), chemical substances, and naturally occurring hormones. It is difficult for a single investigator or group of investigators to study all these modes of induction simultaneously. We should then not generalize conclusions obtained under one limited set of circumstances to the pathogenesis of neoplasia. It may well be, of course, that the very diversity of the causal factors so far unearthed may lead us to an easier understanding of the intrinsic mechanisms and meanings. They should always be kept in mind.

The history of the study of chemical carcinogenesis is of considerable interest as an exercise in the integration of many disciplines in medicine. The basic observation upon which this field of study is founded is the clinical discovery of Pott[1] that a certain occupational group (chimney sweeps) were getting an unduly high incidence of an otherwise rather uncommon neoplasm (carcinoma of the scrotum). This led, after a delay of 150 years, to the demonstration of the carcinogenicity of coal tar to the skin of the rabbit[2] and soon after that to the demonstration of the experimental carcinogenicity of the pure chemical substances 1,2,5,6-dibenzanthracene and 3,4-benzpyrena. Other steps in cancer research, such as the discovery of the carcinogenic action of x-rays, followed the same pattern: the clinical observations by Frieben[3] of skin cancer in x-ray workers being transposed in the experimental demonstration by Clunet[4] some few years later. The induction of neoplasm in animals by ultra violet light[5] was a confirmation of the clinical impression that sunlight was an important factor in the origin of skin tumors in man.

The important field of endocrine control of cancer can be said to be largely founded upon the clinical observation of Beatson[6] that ovariectomy had a salutary effect on certain cases of human mammary carcinoma.

It is clear that the possibility of further correlations at this same level emerging have by no means been exhausted. It is to be hoped that present tendencies

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to "compartmentalize" cancer research will not allow us to lose sight of the common factors concerned.

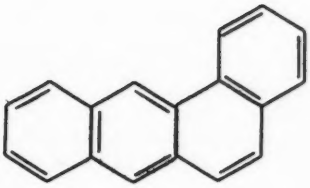
It may be, as many think, that cancer is a problem in abnormal growth: that its solution awaits a complete understanding of normal "growth." This concept does not somehow fit the fact that neoplasia is demonstrably a disease process related to chemical, physical, and viral injury—in animals and men—as such, and that even without knowing the mechanisms involved, it could, at least in part, be effectively controlled by preventive medical measures.

Since the demonstration of the carcinogenic action of the polycyclic hydrocarbons, some 25 years ago, a large number of related and unrelated chemical compounds have been found capable of inducing tumors in animals.

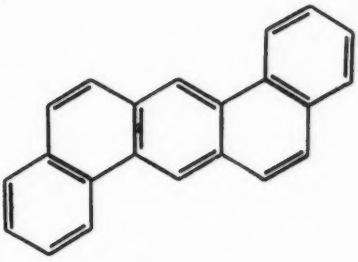
A survey of the substances tested for carcinogenic action by Hartwell[7] in 1947 lists some 340 pure chemical substances with this property. Since that time many more substances have been tested and at least 200 additional substances may be added. Superficially it would appear that if 600 compounds can induce the process, and these are being added to at the rate of almost fifty substances a year, almost anything might be carcinogenic. In fact many of the 600 compounds are closely related, more than 200 being polycyclic hydrocarbons, and a further 100 or so are azo dyes. Much research was devoted to relating chemical structure to carcinogenic potency in an effort to gain an insight into the biochemical mechanisms involved. To this end many chemicals closely related to carcinogens have been synthesized. For example, the benzanthracene nucleus first attracted considerable interest. 1,2-benzanthracene (I) was found to be a border-line carcinogen[8], and yet substitution on this molecule of a benzene ring in the 5,6 position led to the formation of the potent carcinogen 1,2,5,6-dibenzanthracene (II). Workers in the field then proceeded to synthesize all the mono and dimethyl derivatives of 1,2-benzanthracene, many of which are carcinogenic though varying considerably in potency. Substitution at the 5,6 and 9,10 positions seemingly gives rise to 9,10-dimethyl-1,2-benzanthracene (III) and 5,9,10-trimethyl - 1,2-benzanthracene (IV), thus far the most potent carcinogens synthesized by any criteria. This series of syntheses alone accounts for over 150 compounds that are included in the list of known carcinogens and illustrates the misleading impression that is gained by merely adding up the numbers of known active compounds. Several parallel series of closely related hydrocarbons with carcinogenic action have been synthesized. Notable amongst these is 20-methylcholanthrene (V), which was first made by a degration of naturally occurring bile acid, the discovery of which led to speculations about disturbed steroid metabolism as a possible source for carcinogens in the body[9]. These speculations have now been abandoned in the absence of the demonstration of any biochemical mechanism that could accomplish such transformation *in vivo*. The possibility that cholesterol (VI) could be carcinogenic has been a subject of controversy for many years, and recently Hieger[10] has re-opened this subject with a paper claiming the induction of tumors with a particularly carefully purified sample. Up to this time, the claims to the carcinogenicity of cholesterol have generally been invalid, owing to the presence of impurities that are particularly difficult to remove from this substance, even by repeated re-crystallization. Kennaway[11] recently commented that the carcinogenic materials in this range of activity may well be of great importance.

The group of workers in France directed by Lacassagne have been particularly interested in the benzacridine series of compounds (VII, VIII) and have synthesized a series comparable to the benzanthracenes, finding that substitution at one location enhances carcinogenicity and at another depresses it. Many similar compounds with but little variation in biological activity such as the benzfluorenes (IX), the benzcarbazoles (X), and others have been sythesized.

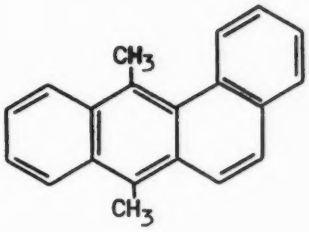
Having made and tested such a large series of compounds the problem of correlating the data obtained arises. This is made extremely difficult by the nature of the biological response obtained. The



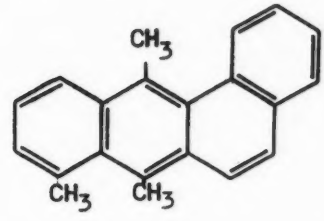
I. 1,2-Benzanthracene



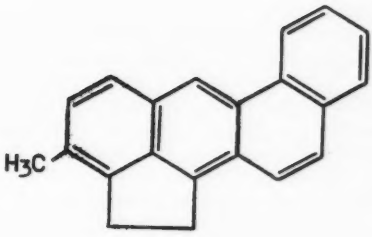
II. 1,2,5,6-Dibenzanthracene



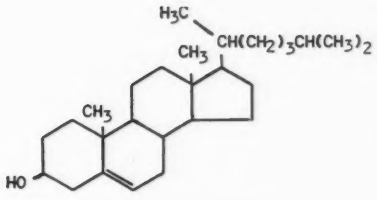
III. 9,10-Dimethyl-1,2-Benzanthracene



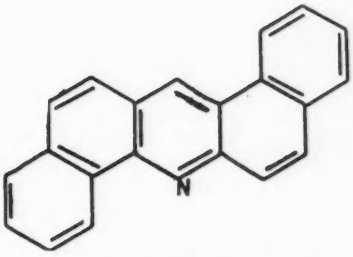
IV. 5,9,10-Trimethyl-1,2-Benzanthracene



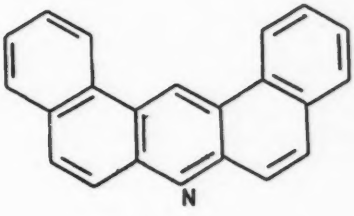
V. 20-Methylcholanthrene



VI. Cholesterol



VII. 1,2,5,6-Dibenzacridine



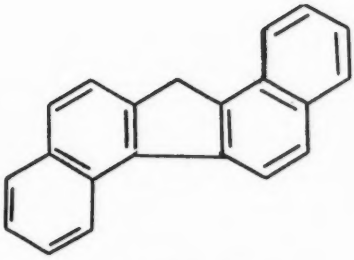
VIII. 1,2,7,8-Dibenzacridine

compounds described have the common characteristic of acting upon the tissue with which they are placed in direct contact; thus when painted on the skin they induce hyperplasia, then papillomas, and some of these papillomas become malignant squamous cell carcinomas. Occasionally a carcinoma arises *de novo*. When injected subcutaneously they will give rise to sarcomas, arising generally from the wall of the cysts formed by the injection of the test material in an oily solution. Tumors may be induced by these agents in a variety of other tissues, such as the brain, bone, or the gut, by direct injection[12, 13]. When they are fed, they give rise, in rodents, to squamous cell tumors of the forestomach but generally not to adenocarcinoma[14]. For tumors to be induced by skin painting, it is generally necessary to apply the substance repeatedly, although single large doses are sufficient in certain instances[15]. For the induction of tumors in other sites only a single injection is necessary, and this may be extremely small, as little as 0.0004 mg. of 1,2,5,6-dibenzanthracene having induced a sarcoma[16]. The response to these agents will vary in two ways: first, in the total number of tumors induced with a certain dosage, and secondly with the latent period of tumor induction. In general, with the more potent of the substances, papillomas will begin to appear on the skin of mice after 10 weeks or so and most of the mice will have carcinomas by the 20th week. Sarcomas, following subcutaneous injection, will arise after 4 to 6 months.

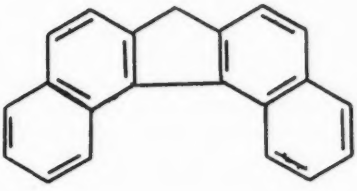
There have been a series of efforts to devise indices indicating carcinogenic potency so that a chemical series could be compared[17, 18]. These indices are all beset by two unfortunate characteristics of these compounds. The first of these is species variation. A particular compound may be particularly potent in one series and yet have almost no activity in another. Secondly, a particular compound may be particularly active when painted on the skin but will exhibit far less activity when injected subcutaneously. When compiling a list of compounds in their order of activity, it is possible to be most misleadingly arbitrary by selecting a particular site or species. Some workers have undertaken complex physicochemical calculations of ionic densities at certain locations of the carcinogen molecule and correlated these figures with carcinogenic potency[19, 20]. These correlations seem impressive until the biological variations are taken into account.

Not only do the quantitative potencies of carcinogens vary from species to species, but the qualitative response may be seen to be different too. Thus injections of methylcholanthrene in the guinea pig will result in the induction of many liposarcomas[21], but although the skin of the rabbit is particularly sensitive to the carcinogens, there is only one recorded instance of the induction of subcutaneous sarcoma in this animal by a chemical[22]. The induction of an osteogenic sarcoma in the monkey by injection of methylcholanthrene, which has recently been recorded, is of interest in view of previous failures to induce tumors chemically in this species[23]. Thus far the number of species responding to carcinogenic chemicals is very large, including salamanders, mice, rats, cats, dogs, etc. The time variation in the induction of tumors in the various species is commonly equated with their life span, although a cursory examination of available data does not support this in all cases. Using 9,10-dimethyl-1,2-benzanthracene, for example, tumors are induced in the rabbit more rapidly than in the mouse in spite of a life span of more than double in the former[24].

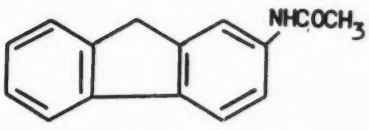
In addition to the easily observed local action of this series of carcinogens, they also have a systemic action noted under special laboratory conditions. Murphy and Sturm[25] first noted that when certain mice were treated on the skin with tar, not only were skin tumors induced, but there was a concomitant increase in the number of lung adenomas seen as well. This phenomenon has been well studied since, and may be particularly noted in strains of mice having a high incidence of pulmonary adenomas such as the Strain A. The administration of a hydrocarbon carcinogen to such animals will speed up the time of appearance of these tumors and will greatly increase the total



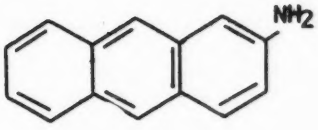
IX. 1,2,5,6-Dibenzfluorene



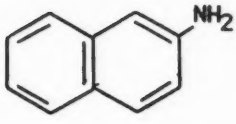
X. 3,4,5,6-Dibenzcarbazole



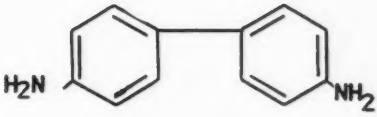
XI. 2-Acetylaminofluorene



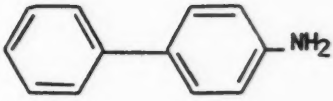
XII. 2-Anthramine



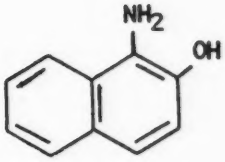
XIII. B-Naphthylamine



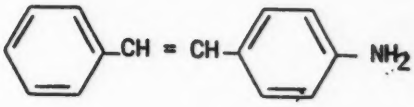
XIV. Benzidine



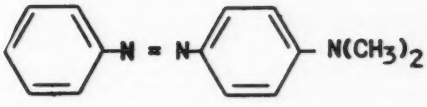
XV. 4-Aminobiphenyl



XVI. 1-Amino-2-Natphthol



XVII. 4-Aminostilbene



XVIII. p-Dimethylaminoazobenzene

number seen. Some investigators find that the induction of lung adenomas, usually following the intravenous injection of a small amount of a suspension of carcinogen is a particularly accurate index of activity[26, 27]. The induction of leukemia by the skin application of a hydrocarbon carcinogen is again a well established procedure in the mouse; it is often the custom to apply the carcinogen to the skin at various sites so that few skin tumors will arise and the carcinogen will manifest its systemic effect without interference[28].

There is another series of important carcinogenic agents that differ from the polycyclic hydrocarbons described above in that they exert their action primarily when administered by mouth. This group of carcinogens is very diversified including aromatic amines, azo dyes, and other compounds that will be considered separately.

The discovery of the activity of the compound 2-acetylaminofluorene (XI) marks a somewhat new departure in the unearthing of these agents. This compound, synthesized as an insecticide, was tested for chronic toxicity for two years prior to introduction into general use by feeding to rats by the team of scientists at the United States Department of Agriculture[29]. These investigators discovered that this substance induced a large variety of tumors in the animals under treatment, and this potent carcinogen was, therefore, never released as a commercial product. This new pattern of protection of society from possible carcinogens has become particularly important as more and more synthetic materials are released for human application. The specific design of chronic toxicity tests to include a study of carcinogenic action has not yet received adequate attention, but this will undoubtedly emerge in the near future.

The tumor induction pattern seen when acetylaminofluorene is fed in the diet at a level of some 0.3% is rather curious: the tumors induced have the common characteristic of arising largely from tissues of ectodermal origin. Thus tumors occur in the liver, bile ducts, breasts, bladder, intestine, brain, external auditory canal, and other situations. This carcinogen is notable for the wide number of animal species in which it will induce tumors including the mouse, rat, cat[30], dog[31], and rabbit[32]. The latent period of tumor induction is variable from six months in the mouse to about three years in the dog, with the tumors in the cat occurring early. It would seem likely that this carcinogen operates through an intermediary metabolite, though this has not yet been detected. Although this compound is not active when painted on the skin, the very closely related 2-anthramine (XII) does have this property. Unfortunately the latter's high degree of toxicity has prevented more detailed study[33].

Other aromatic amines, namely beta-naphthylamine and benzidine (XIII, XIV) have a much more localized action in the animal, inducing only tumors in the urinary tract. These substances were discovered primarily as a result of statistical studies of workmen in the aniline dye industry who were known to have unduly high incidence of bladder tumors. It was demonstrated[34] that beta-naphthylamine could induce bladder tumors in the dog, and at a later date, when it was thought that other carcinogens still remained unaccounted for, that benzidine[35] also had this property. In more recent years a third carcinogen, 4-aminobiphenyl (XV), is also thought to be implicated in the induction of bladder tumors in man[36]. Studies by Bonser and her co-worker[37] of the metabolism of beta-naphthylamine mark a cornerstone in studies of carcinogenesis. These investigators have found that beta-naphthylamine itself is not directly carcinogenic to the epithelium of the bladder when implanted into it in the form of a pellet. However, when the urinary metabolite of this carcinogen, 1-amino-2-naphthol (XVI), is implanted into the bladder, tumors are induced. There are, as yet, no reports on the carcinogenicity of this compound in tissues other than the bladder.

4-aminostilbene (XVII) and some related compounds have a carcinogenic action closely comparable to 2-acetylaminofluorene. This substance when fed

by mouth induces tumors in a variety of tissues (liver, intestine, external ear duct, *etc.*). There is less data pertaining to the action of this group of substances than for the others owing to their more recent discovery[38].

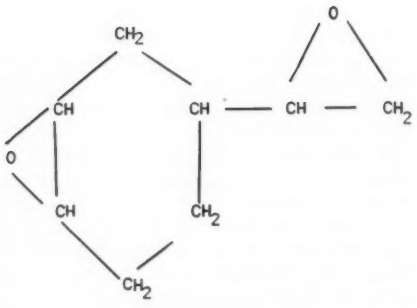
The incidence of many liver tumors amongst the Bantu natives of South Africa and also amongst the Javanese has aroused considerable interest in recent years[39]. Various explanations have been advanced for the localized occurrence of these neoplasms, one of which being the dietary deficiencies that are invariably associated with cirrhosis has been particularly popular. Certain other investigators decided to concentrate on a search for specific liver carcinogens that might be peculiar to the diets of these races. As a result of such studies, it has been found by Hoch-Ligetti that liver tumors may be induced in the rat by feeding them with quantities of red Chile peppers[40]. Cook and Schoental[41], concentrating on the Bantu problem, discovered that certain senecio alkaloids are carcinogenic. These particular plants are used as medications by the Bantus, and their use as medication for children is also widespread in the West Indies. Thus far, the pure alkaloids retrorsine and isatidine have been found capable of inducing liver carcinoma in the rat following feeding. It may well be that a correlation between this disease incidence and these substances does in fact exist, although more field work and corroborative human data is necessary before any conclusions can be derived.

The remaining carcinogens to be discussed belong to a diverse group of chemical structures and were all tested as carcinogens on an experimental basis for one reason or another, but in this instance there was no clear connection between human usage and the experimental demonstration.

The Japanese investigators Kinosita and Yoshida[42, 43] discovered that the azo dye p-dimethylaminoazobenzene (butter yellow) (XVIII) when fed to rats induced hepatomas. Since that time a large variety of azo dyes, perhaps 100 or more, have been synthesized and some found active, others less so, and some inactive. Once again this group of substances accounts for a large number of the positive carcinogens reported. A great deal of work has been carried out on the influence of diet on the tumors induced by these compounds and on their biochemical behaviour. That will be discussed elsewhere. It is notable that certain of these substances have been in contact with man in the diet and as coloring agents elsewhere for many years, but no connection to tumor induction has ever been noted or suspected.

Another group described recently as carcinogens are the epoxides (XIX), a group that is chemically quite dissimilar to previous carcinogens. So far the literature on these agents which induce tumors locally in the rat following subcutaneous injection is quite limited[44] and it would be of great interest to have further work performed with them. In recent studies of the carcinogenic potentialities of atmospheric pollutants, Kotin[45] has found that certain fractions of air dirt, free from aromatic hydrocarbons, are nevertheless carcinogenic. He has suggested that these epoxides, known to occur in the atmosphere of urban areas, may account for this. This work is quite preliminary but of some interest.

Several substances that are primarily of interest as cancer chemotherapeutic agents have been found to be carcinogens during the past decade. First amongst these is the hypnotic, urethane (XX), a substance that until recently was a popular mild sedative in children. The activities of urethane are enormously diverse including its action on the nervous system, a specific cytotoxic action on the lymphocyte, and a specific carcinogenic action on the lung. Urethane will induce tumors in the lung of the mouse[46] and the rat[47] when administered either in the drinking water, or by intraperitoneal injection. The tumors induced are the common variety of adenoma seen in the rodent that are truly without any clear counterpart in human pathology. In a recent report[48], Rogers, using a technique of embryonic tissue transplantation, has demonstrated that this substance does not act directly on the lung, but is metabolized after a very short time by the



XIX. 4-Vinylcycohexane Dioxide

$NH_2COOC_2H_5$

XX

URETHANE

Nitrogen Mustard (HN_2)

$CH_3\text{-}N(CH_2CH_2Cl)_2$

XXI

METHYL di(2-CHLORETHYL)AMINE

animal to an active carcinogen. This experiment will be discussed further in another article. Again, although urethane only induces tumors in the lung when administered alone, it has been demonstrated[49] that it can initiate a latent tumor change in the skin following painting that will be revealed by the subsequent addition of a promoting agent.

Another well known chemotherapeutic agent, nitrogen mustard (XXI), has a carcinogenic action that is in many ways similar to that of urethane. In thorough investigations by Heston[50], both the nitrogen and sulphur mustards have been shown capable of inducing lung adenomas. In these studies the mustards were introduced in minute doses by intravenous injection into the tail vein of the mouse. Certain other investigators have claimed a local carcinogenic action for the mustards[51, 52], but these studies were somewhat small and cannot be considered as substantiated without further work.

Beryllium in various forms, such as the oxide, sulphate, or zinc silicate, has been extensively studied[53, 54]. Osteogenic sarcomas are produced following intravenous injection, and Vorwald induced tumors classified as bronchogenic carcinoma in rats and rabbits following the inhalation of beryllium oxide[55]. This latter demonstration is particularly striking in view of the importance of bronchogenic carcinoma in man. However, no neoplasms have been recorded in the human following exposure to beryllium, although it is possible that man has not yet been exposed long enough to hazards of beryllium for a carcinogenic action to have become manifest.

Two other metals, namely chromium in the form of chromates[56] and nickel in the form of nickel carbonyl, have been suspected for some time as possible causal factors in bronchogenic carcinoma and in carcinoma of the ethmoid sinus in the human. So far chromium has not proved to be carcinogenic in the laboratory, but a recent report claims the induction of sarcomas in rats with nickel[57]. Arsenic[58] has long been considered to be a possible cause of certain human cancers; again little confirmation of this has been forthcoming from laboratory studies with the exception of the induction of a few papillomas in the skin of the rabbit[59, 60]. This aspect of carcinogenesis cannot be considered as properly investigated as yet, and it is likely that further studies would reveal results of some importance.

Perhaps one of the most surprising

discoveries in carcinogenesis in recent years is that concerning the action of cellophane and related substances. Oppenheimer, while conducting studies on hypertension, wrapped the kidneys of the rat with cellophane to induce a fibrous constriction of the renal artery. He found that fibrosarcomas could be induced in this manner. In further studies, sarcomas were induced in more resistant animals, such as the C 57 black mouse, and the specificity of the phenomenon was shown to be confined to a certain number of plastics of a particular chemical nature[61]. Contrary to initial impressions, it would seem as though these substances are not inert, but are metabolized by the organism.

Lastly a series of experiments in which reticuloses of various sorts have been induced in the rat following continued administration of trypan blue must be mentioned[62]. Trypan blue is a somewhat impure mixture when obtained commercially, and as it has been pointed out[63], the experiments demonstrating its action by the Gilmans do not in any way pinpoint the responsible chemical substance. The lesions induced by this substance in the rat consist of a variety of neoplasms of the lymph nodes and reticulo-endothelial system generally. The authors have likened some of these lesions to Hodgkin's disease in man, though doubtless much room remains for debate on the precise nature of these lesions.

This brief summary is intended to illustrate the variety of the chemical carcinogens known to us at present; to describe the range of their activity, and the use that may be made of them for further research. It is of considerable interest that the larger and more potent groups of carcinogens have become known by a combination of experimental research founded on astute clinical observation. The greatly increased interest in environmental cancer associated with extrinsic chemical factors of the present time will doubtless reveal more carcinogens. A good example of that is in the much vexed question of the role of cigarette smoking in lung cancer. A carcinogenic tar has been obtained from cigarette smoke that induces tumors in the skin of mice[64]; thus far small amounts of benzpyrene, thought to be inadequate for tumor induction by some, have been detected in this material. It seems as though this material undoubtedly contains other carcinogens, and it is the somewhat difficult and long term task of the experimentalist to determine what these may be. It is, of course, a valid question to ask whether the induction of skin tumors in the mouse can have any bearing on the induction of epithelial tumors in the bronchus of man: undoubtedly the two phenomena may be unrelated, and only further experimentation will tell us whether or not this is the case. The call for the inclusion of a test for carcinogenicity of new materials of a synthetic nature to be used over a long period by man is clearly indicated, although it is still difficult to design such a test in a comprehensive fashion.

As it was stated earlier, there are approximately 600 chemical compounds with carcinogenic activity; clearly all of these have not been described. However, the general headings discussed do include the large majority of the basic structures concerned.

The basic research into the biological and chemical aspects of carcinogenesis will be considered in the two accompanying papers. To conclude this summary, mention should be made of the theories that have been proposed of a general nature concerning carcinogenesis and neoplasia. Haddow[65] demonstrated that animals bearing transplanted tumors would suffer an inhibition of tumor growth from the injection intraperitoneally of certain of the carcinogenic hydrocarbons: testing a large series of carcinogenic and non-carcinogenic hydrocarbons, he found that there was a correlation between the carcinogenicity of the compound and its tumor growth inhibiting property. He therefore proposed a theory to the effect that carcinogenicity and growth inhibition invariably went hand in hand. To this end, he conducted tests for carcinogenicity on substances known to inhibit growth and tests for growth inhibition (in fact, for tumor chemotherapy) of substances known to be carcinogenic. As a result of these

hypothetical proposals, the aminostilbenes were found to be carcinogenic and urethane was found to be a chemotherapeutic agent of value in human leukemia. The somewhat striking fact that urethane, the mustards, and x-rays all are both carcinogenic and tumor therapeutic agents certainly seemed to warrant the construction of a theory. However, reinvestigation of the intrinsic nature of of the inhibition of the transplantable tumor by hydrocarbon carcinogens by Haddow and co-workers[66] revealed that this was by no means a specific effect upon the tumor. It was found, in fact, that the carcinogen in the doses given was merely poisoning the animal by way of inhibition of protein metabolism and that it was possible to induce a similar inhibition by restricting the diet. The phenomenon of dietary restriction, inhibiting both tumor growth and the induction of tumors, has been extensively studied by Tannenbaum[67], and often explains many otherwise curious findings. The theory originally proposed by Haddow has now been abandoned, though it remains of particular interest in that in spite of its basic unsoundness, it was the means of unearthing new carcinogens and a chemotherapeutic agent.

The most discussed general theory of cancer in the present century is the "Somatic Mutation Theory of Cancer." This hypothesis, most commonly ascribed to Bauer[68], suggests that a mutation occurs in a somatic cell and that this gives rise to a neoplasm. At the time of its origin, it was already known that x-rays and ultraviolet light could both give rise to mutations and to cancer; subsequently it was demonstrated by Auerbach that mustard gas, a chemical, could induce mutations in Drosophila, and eventually it was found that nitrogen mustard and sulphur mustard could induce lung tumors in the mouse. Lists of substances have now been drawn up in which mutagenic agents are shown also to be carcinogenic by the proponents of the theory[70] and other lists in which many mutagens are listed that are not carcinogenic by the opponents of the theory[71]. The chief valid objection to the theory, is that it has little practical meaning and that, by its very nature, is not amenable to proof. Haldane[72] has pointed out that the only method known to us of demonstrating the occurrence of a mutation is by a back cross: somatic cells by definition cannot be crossed and therefore the theory can never be proved. Genetics, however, is rapidly changing and biochemical meaning is now being given to previously mysterious occurrences. The definition of a mutation in the strict sense has been severely modified already by the bacteriologist. It may be that this concept will serve some useful purpose.

More useful than the search for proof of hypothesis such as this, seems to be the continued study of the mechanisms of action of the known carcinogens with all our techniques, and the study of the intrinsic nature of the pathological reaction known as neoplasia. These chemicals, as previously pointed out, are only one of the sets of factors resulting in neoplasia: viruses, ionizing radiations of various sorts, and hormonal imbalances play as important a role. We are, it would seem, dealing with a general pathological reaction of the tissues to a whole variety of diverse agents. It is a reaction as standardized as inflammation to almost as diverse a series of stimuli. So far it is a reaction without meaning, but this no doubt will become apparent. For the time being, it would seem as though precise observation will yield more than hypothetical contemplation.

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BIOLOGICAL STUDIES IN CARCINOGENESIS*

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This brief review is devoted to a consideration of the use that has been made of carcinogenesis as a technique for the study of neoplastic change as a dynamic process. Previous to the knowledge that tumors might be induced or transplanted under controlled conditions in the laboratory in a variety of ways, it was only possible to study spontaneous tumors, mostly in man, and most often as autopsy specimens. Although a great deal of clinical knowledge has been accumulated over the years relating eventual malignancies to early, so-called precancerous lesions, the bulk of neoplasms are only described in detail after they have become well defined malignancies, and generally their course and life histories are matters for conjecture alone.

A major aim of cancer research is, of course, a complete understanding of the pathogenesis of neoplasia. It has become self-evident during this century that this is no easy matter, and a science of cancer research has evolved with many divergent branches and disciplines. Some of the approaches taken towards an elucidation of these problems go directly to investigations of intricate intracellular mechanisms: namely, cytogenetics and enzymology. Thus far, however, the results obtained in these fields, though of immense interest and importance in their own context, have yet cast no new light on cancer. Other, somewhat more obvious approaches have attempted to use the discovery of the artificial induction of tumors to elucidate some clinical impressions; some clarification, and most important, direction for clinical research has been gained.

Three major areas of study will be considered: first, studies of the histological appearance and genesis of the earliest lesions seen following the application of carcinogens; secondly, the influence of disturbances both physiological and pathological, other than carcinogenic, on the process of tumor induction; and thirdly, the nature of the life history of neoplasms when studied from their earliest inception to their termination as seen under laboratory conditions.

Studies of Experimental Precancerous Tissues

For obvious reasons, the skin has been found to be by far the simplest tissue to use experimentally in studies of the early effects of carcinogens. It is possible to apply carcinogenic hydrocarbons to the skin of mice at a dose that will eventually induce malignant tumors in all the treated animals. Such skin is, therefore, known to be truly precancerous. Many investigators have studied such tissues histologically in an effort to pinpoint changes that occur that may be said to be specifically associated with the future emergence of neoplasm. The initial effects of a carcinogenic hydrocarbon consists of some damage to the skin, seen as inflammatory change in the epidermis and dermis, with perhaps necrosis of some of the cells; there is a hyperplasia of the epidermis within a very short time (48 hours) with an attendant increase in the mitotic rate. In several studies of a series of related compounds, some of which were carcinogenic and others not, Pullinger[1] has found that the carcinogens induce an enlargement in the basal cells of the epidermis within the first 2-3 days, and, in particular, an enlargement of the nuclei of these cells that is not seen with the non-carcinogenic substances. This effect is, however, transient and after a matter of a week or so, the two tissues (one treated with carcinogen, the other with a non-carcinogenic irritant) will be indistinguishable. In a somewhat complex study, Glucks-

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mann[2] has compared the early effects of the carcinogen, 3,4-benzpyrene, with that of non-carcinogenic irritants such as turpentine and barium sulphide. He finds that the hyperplasia induced by the carcinogen differs from the non-specific variety in that the distribution of cells coming from the skin appendages and basal layer of the epidermis is different in these cases. This differentiation is once again a matter of the dynamics of the situation, and also necessitates a high degree of skill in differentiating epidermal elements on a histological basis that is not acceptable to some histologists. More recent studies by Salaman[3] and Gwynn have found similar differences in the pattern of hyperplasia induction by benzpyrene and the vegetable mixture, croton oil. In several studies, Orr[4, 5] has compared the effects of carcinogens and non-carcinogens on the epidermis of the mouse; he has observed some of the changes mentioned by Pullinger, and in addition, has drawn particular attention to changes occurring on the dermis in the area subjacent to the carcinogen painting. These dermal changes have been more recently commented on in the studies of Ma[6]. Orr's interest in the changes in the dermis beneath the epidermal carcinogen treated area stems primarily from clinical observations of such changes beneath epidermoid carcinoma in the human. It is Orr's belief that the dermal changes exert an important role in the eventual origin of tumors of the skin, and that probably the effect of such damage is to give rise to local anoxia. To confirm this impression, he performed some experiments in which adrenalin injections were combined with the application of carcinogens, and others, in which threads were inserted through the dermis in the painted area, inducing local fibrosis; both these procedures were found to increase the numbers of tumors induced. A re-investigation of these experiments by Ritchie[7] failed to reveal such a potentiating effect. More recent studies by Orr, using skin grafts treated with carcinogens, have shown that the graft bed from which the treated epidermis has been removed still contain potentialities for the emergence of epidermal neoplasms. This discovery is extremely revolutionary in its implications. These experiments, however, should await further repetition before final judgment is made. In a very large series of studies by a group of investigators under the direction of Cowdry, every aspect of epidermis treated with the carcinogen 20-methylcholanthrene has been studied. Histological studies have compared such treated tissue with tissue following wounding, and many histochemical investigations and cytological studies have been carried out. These experiments have been summarized by Cowdry[8] and once again, some of the early enlargements of cells and nuclei are noted, as well as some alterations in mineral content that cannot be considered entirely specific.

It must be noted in all these experiments that the action of one or two known carcinogenic hydrocarbons has usually been compared to the effect of one or two non-carcinogenic irritant substances or perhaps to the effects of wound healing. In order to establish a specificity for the changes seen with the carcinogenic hydrocarbon in its early stages, it would be most desirable to compare it with a variety of other damaging agents and procedures. The only changes distinguishing the carcinogens from the small number of other factors studied has been a transient cellular and nuclear enlargement. This change, it must be emphasized, can only be seen when the tissues are studied in a dynamic fashion: that is, when daily sections are taken. When static studies on the skin are made with present histological methods, it would seem impossible to distinguish between carcinogen treated skin and any other hyperplastic skin.

A series of investigators have taken advantage of the fact that many of the carcinogenic hydrocarbons are intensely fluorescent materials in order to study their early distribution within the cell by fluorescence microscopy. The earliest of these studies was carried out by Graffi[9] using benzpyrene, and subsequently by several Scandinavian investigators and others[10, 11, 12, 13]. All these investigators

have been struck by an accumulation of the fluorescent material around the nucleus of the epidermal cells suggesting that it may localize in the mitochondrial elements. Again it must be pointed out that these carcinogenic hydrocarbons are very rapidly metabolized to non-fluorescent materials, and it may well be that an undetectable metabolite is formed immediately; that it finds its way to the nucleus and is, in fact, more important in the origin of subsequent changes than the more easily detectable portion. These investigators have again commented upon the cytological changes of a transient nature seen by others. In more recent years, the fluorescence technique has been severely criticized by some of these workers who are divided in their interpretations (Norden-Setala[14,15]), and will probably be superceded by techniques using isotope labeled materials and the technique of radioautography. As yet such data is not available. It would seem clear from these studies that no clear histological evidence of precancerous change can be expected to be located in early hyperplastic lesions not manifesting other gross biological changes. It is to be hoped that newer techniques, such as radioautography and electron microscopy, will eventually assist us in this respect and tell us, at the least, where to look for the changes.

In recent years, carcinogenesis in animals has become more and more used as a practical procedure in the elucidation of environmental carcinogens in the human. There has, therefore, been an understandable desire to introduce some quicker method of testing than is afforded by the induction of actual tumors, a process taking anywhere up to two years. Suntzeff and others[16] have suggested the use of a so called "sebaceous gland test" on the skin of the mouse. These investigators find that skin treated with a carcinogen has an absence of sebaceous glands, and have described a new method of sectioning the skin so that the glands may be counted easily. This method has not been adequately investigated with many non-carcinogenic irritants thus far, and is likely to suffer from the disadvantage that many carcinogens will not show a positive result in one species, but will in others. In the view of the author, data now at hand illustrate that the method is not likely to be reliable.

An interesting technique for the rapid induction of tumors is that of using embryonic tissue fragments transplanted subcutaneously with some of the carcinogenic material. These procedures are carried out in pure strain mice to obviate the genetic difficulties in transplantation. This technique has been used extensively by Smith, Green, and Horning[17,18,19] primarily with the purpose of inducing tumors in tissues otherwise difficult to bring into contact with carcinogens. Thus Horning has induced a series of prostate tumors, and a variety of tumors have been reported by Smith. Although a certain number of tumors do occur very early with this technique, some do not occur until after almost a year. In a recent and very striking experiment, Rogers[20] has found that it is possible to expose embryo lung tissue to the carcinogen urethane *in vitro* and then implant it subcutaneously without inducing tumors; however, if this same lung tissue is exposed again *in vitro* to the blood of a rabbit previously dosed with urethane, it will become neoplastic following transplantation. From this experiment it would seem likely that urethane itself is not directly carcinogenic to the lung tissue, but that it is metabolized to an active carcinogen in the animal. This experiment, is of course, particularly striking from the standpoint of basic implications in the mechanism of action of the carcinogen; additionally, however, it also points to the inadvisability of using this method for testing suspect carcinogenic materials. It seems quite possible that embryo grafts may be left in contact with a suspect material and yet not be transformed into neoplasms through the inability of the organism to metabolize the precursor into the true carcinogen.

The study of the early lesions induced by carcinogens in sites other than the skin is less common, owing to the practical difficulties arising from their pres-

ence in less accessible sites. The histogenesis of the tumors induced by subcutaneous injection of carcinogen has been studied and the tumors have been found to arise, to some extent, from the fibrous tissue of the wall of the cyst formed by injection of the carcinogen, usually in an oily medium[21]. Tumors induced in the intestine and lung of the experimental animal thus far have less interest to the human pathologist owing to the difference both in the normal anatomy and neoplasms in the commonly used laboratory animals. A great deal of effort has been expended on the study of carcinogenesis in the intestine, but the results have thus far been somewhat disappointing. The gastro-intestinal tract of the rodent differs from that of the human in having a fore-stomach, a squamous cell lined part of the stomach. When carcinogens are introduced by mouth in the mouse, the largest number of tumors to be induced will occur in the squamous portion of the stomach where a series of hyperplastic changes leading to papilloma formation and eventually to carcinoma will be seen. These changes are the same as those occurring in the skin following painting. A small number of adeno-carcinomas are induced by feeding the hydrocarbons, but generally these may only be induced by injecting the carcinogen into the submucosa directly. Early changes with this procedure have not been studied in the same detail as those from painting and it is felt that the procedures used are intricate enough to make interpretation difficult. The results of these experiments in carcinogenesis will illustrate clearly that although the glandular portion of the intestine is quite sensitive to the action of the hydrocarbon carcinogens, it is also well protected, presumably by mucus secretion, from the action of the substances when fed by mouth.

The histogenesis of the lesions arising from the feeding of the carcinogen acetylaminofluorene have not been as well studied as those in the skin with the exception of tumors occurring in the external auditory duct, and to some extent the tumors occurring in the liver. Skoryna[22] has shown that the tumors in the external auditory canal of rats commonly seen with acetylaminofluorene, 4-aminostilbene, and certain other carcinogens arise from a gland in this area that is commonly infected in the affected animals, and that this tumor is not likely to occur in the absence of infection. With both acetylaminofluorene and the azo dye carcinogens, such as p-dimethylaminoazobenzene, hepatomas and cholangiomas occur; these tumors are invariably preceded by the occurrence of cirrhosis of the liver. The association of cirrhosis of the liver and hepatoma in man is well known[23]. More recently the Seneccio alkaloids have been found to be liver carcinogens, and the suggestion has been put forward that these substances can induce hepatomas in the absence of previous cirrhotic change.

The Role of Certain Extrinsic Factors in Carcinogenesis

At the time of the discovery of the carcinogenic action of X-rays[24], and later of coal tar[25], one of the more popular theories for the origin of neoplasia was the "Theory of Chronic Irritation" ascribed most commonly to Virchow[26]. The action of x-rays and coal tar, inducing all the manifestations of so-called "chronic irritation" were held by many to be good supporting proof of the validity of this view point. Subsequent to the isolation of a single component of coal tar, 3,4-benzpyrene, considerably less an "irritant" than the whole mixture and yet potently carcinogenic, the theory that chronic irritation might account for the emergence of neoplasia in itself was dropped by most investigators. The possibility that trauma, acute or chronic, might exert some secondary effect in the origin of tumors was in the mind of certain investigators. Cohnheim[27], who was opposed to many of the views of Virchow, suggested many years ago that trauma and its attendant hyperemia might occasionally cause certain latent tumor rests to grow into active neoplasms. The advent of tar carcinogenesis permitted the experimental investigation of the role of trauma in carcinogenesis. In several experiments, Deelman[28,29], using mice, found that combining simple cutting

trauma with tarring increased the speed at which tar tumors were formed, increased their numbers, and had a tendency to localize them to the site of the injury. These experiments were not too well accepted during the decade in which they were performed, although they were extended to some extent by Cramer, who found that cutting could increase the tendency for induced skin papillomas to become malignant. Repetition of the experiments of Deelman some twenty years later by Pullinger[30] provided confirmation of the original conclusions, although differing from them quantitatively in finding that the effects of the trauma were relatively small. An extensive reinvestigation and extension of this work, by Rous and his colleagues[31, 32], using the rabbit instead of the mouse, provided ample confirmation of a role for trauma, and to some extent clarified its place in the mechanism of carcinogenesis. Rous found that the skin of the ear of the rabbit could be tarred or painted with methylcholanthrene until a number of papillomas had appeared, and that these papillomas would then regress if the treatment were stopped. These same tumors could then be made to re-appear in precisely the same location by either a resumption of the carcinogen treatment or instead by wound healing, the wound healing being effected by punching a hole through the area with a cork borer. Certain other non-carcinogenic agents, such as turpentine, could be used instead of the wounding or carcinogen treatment to effect the re-appearance of the tumors. The tumors that regressed and then reappeared were studied carefully and found to be mostly papillomas, although some, called "carcinomatoids" had the appearance of malignancy in that they were invasive, but had a tendency to behave in a classically unmalignant fashion; these tumors are somewhat reminiscent of the recently described keratoacanthomas in man that appear to be malignant but often regress. From these experiments, Rous concluded that tar or methylcholanthrene initiate far more "Latent Tumor Cells" in the skin than ever became grossly neoplastic and that carcinogenesis could be seen to be a two stage process in which the specific carcinogen initiated the change which could then be promoted into tumor growth either by the same agent or by other stimuli, such as trauma, in themselves non-carcinogenic.

Another series of experiments in which chemical irritants, rather than wound healing, were used paralleled the experiments of Deelman and Rous and led to identical conclusions. Berenblum has summarized most of the earlier data on this work in a review entitled "Irritation and Carcinogenesis"[33]. The most outstanding demonstration was that a vegetable mixture, croton oil, could be added to a weak solution of the carcinogen benzpyrene and would enormously potentiate its effect[34]. Mottram[35], in an effort to elucidate the nature of this phenomenon, demonstrated that the skin of the mouse could be painted but once with a solution of benzpyrene in a dose too low to induce tumors and that tumors would then be induced by subsequent applications of croton oil. Further repetition and extension of this observation[36, 37] showed that application of croton oil prior to the administration of the carcinogen had no effect, but that croton oil could be applied to carcinogen treated skin as long as forty-three weeks afterwards and would still result in as large a yield of tumors as if the croton oil had been applied only three days subsequent to the carcinogen treatment. Several different carcinogens and several different concentrations of the same carcinogen were tested in single applications followed by croton oil, and it was found that tumor induction with a variation in the number of tumors produced occurred with a similar latent period of tumor induction. The tumor induction pattern seen with applications of a single dose of carcinogen followed by croton oil differed significantly from that seen following repeated applications of a carcinogen, in that the number of tumors induced reached a peak after some weeks and remained at this level, usually not more than fifty to sixty per cent of the treated mice developing tumors; with repeated applications of carcinogen, tumors continued to accumulate through the experi-

ment and almost invariably 100 per cent of surviving animals developed tumors if the treatment was continued long enough.

The hypothetical interpretion of these experiments is that the single application of carcinogen gives rise to a certain number of changed cells (latent tumor cells) in the treated tissue (initiation) and that the croton oil subsequently converts these cells into actual tumors (promotion). The reason for the flattening off of the curve of the number of tumors induced to a plateau being that all available changed cells have been promoted into tumors; when a carcinogen is applied repeatedly, more and more initiated cells are produced as well as promoted so that no such pattern is seen.

Croton oil was chosen for testing in this context as one of a series of known irritant or vesicant substances; it was hypothetically used to study the role of chronic irritation in carcinogenesis. However, it soon emerged that many irritant substances[38] had no such action in carcinogenesis, and it is in fact possible to induce considerable hyperplasia in the skin of mice treated once with carcinogen, and yet induce no tumors. Croton oil action thus seems to be specific to the same degree as that of the carcinogen, and to have an action that can certainly not be correlated with that of wound healing on a quantitative basis. It displays considerable species specificity, having no action like that described in the rat or guinea pig, though similar action for it after a longer latent period has been described in the rabbit[39]. No inkling as to the active constituent of croton oil has yet been obtained, although fractionation combined with biological studies are now underway in this laboratory[40]. Many other materials have been tested for promoting activity and the larger percentage have been found inactive; Salaman and Roe have found iodoacetic acid and chloroacetophenone to be active promoting agents, and this observation has been confirmed by Boutwell and Rusch[41], although others have found these substances inactive[42]. Boutwell and Rusch have given a brief report on the promoting activity of a series of agents including quinones and cresols, but this data has not yet been well documented. A recent report by Setala has claimed that Span-20 and Tween-80, emulsifying agents that are commercial mixtures containing largely sorbitan monolaurate or polyoxyethelene sorbitan monolaurate, are as active as croton oil as promoting agents[43]. In the experiments reported, the substances were used in amounts greatly in excess of that used in croton oil experiments, and repetition of this work thus far does not afford confirmation for these conclusions. Setala feels that the detergent property of these substances is concerned in the promoting activity seen and has performed many other experiments lending weight to these conclusions.

Promoting substances have been demonstrated in certain other complex organic mixtures, notably creosote and fractions of "slurry oil" obtained from petroleum catalytic cracking[44]. Certain fractions of petroleum have the curious property of being non-carcinogenic to the skin of the mouse alone, but exerting a promoting effect equal to that of croton oil; these same fractions, however, are carcinogenic alone to the skin of the rabbit. In the case of creosote oil, a basic fraction was found to augment the effects of benzpyrene[45]. More recent studies to repeat and eventually isolate this substance have not found any confirmation for these original observations[46]. This, however, was to be expected in view of the complex nature of creosote and the many variations from sample to sample and in method of preparation.

Many investigations to demonstrate more promoting agents are underway, both in this department and elsewhere; as yet no pure chemical substance with a potency equal to that of croton oil seems to have been found. In the original paper on croton oil[47], it was found that the oil alone induced a minimal number of papillomas. A series of other investigators have also found that croton oil alone may induce as many as one or two papillomas in groups of twenty mice. This observation is difficult to interpret, and would seem to mean that probably

these mice had been accidentally contaminated with a minute quantity of carcinogen (Croton oil will induce tumors in skin treated with one gamma of DMBA [9,10-dimethyl-1,2-benzanthracene])[48], or perhaps that an inherent, latent tumor state occurs to some small extent. The general behaviour of croton oil, its pattern of tumor induction, its failure to induce tumors at all alone in many experiments, distinguishes it so radically from other carcinogens that a special explanation of its behavior becomes necessary. It is now clear that hyperplasia cannot be involved as the mechanism in the promoting phase of carcinogenesis. The explanation of this phenomenon now seems to await the isolation of the active principle of croton oil. Preliminary studies with the fractions of croton oil prepared for identification purposes indicate that the most irritant fractions do not possess promoting properties[40].

There seems to be little doubt that many more substances with promoting action remain to be discovered; as yet the amount of work on this subject has been restricted to a few laboratories. However, the general principles expressed in this hypothetical interpretation have many further implications. First the question arises about the possible existence of initiating agents that do not have the power to promote. Roe and Salaman[49] have found that urethane alone may be painted on the skin of mice and yet will not induce tumors; when this treated skin is treated with croton oil tumors are produced. They have called this substance in this context an "incomplete carcinogen." Other substances, such as the nitrogen mustards, tested by these workers have not revealed any such activity. Tumors may be initiated in the skin by ionizing radiations in doses well below those inducing tumors alone, and therefore classified previously as dangerous. For example, it requires up to 8000 r.e.p. beta to induce skin tumors in the mouse according to some investigators; yet 800 r.e.p. beta can induce a latent tumor change[50] and this is by no means the lowest level that seems to be active[51]. Certain other examples of a two stage mechanism in carcinogenesis have been provided in experiments using remotely acting substances: thus radio-active iodine has been combined with thiouracil to induce thyroid tumors in dosages below those active alone in either case[52]. The systemically administered carcinogen, acetylaminofluorene, has been demonstrated as capable of initiating tumors in the skin, although alone it does not produce tumors in this location[53]. Experiments performed some years ago by Lacassagne and Vinzent[54] demonstrated that abscess cavities in the rabbit could be irradiated with very low dosages of x-rays (well below the carcinogenic dose) only once and would induce malignancies. It thus becomes obvious that the determination of a danger level in carcinogen exposure is a complex affair. In specifying the tolerance level to radiations for example, it is necessary to know many other factors, such as the presence of healing tissue, or conceivably the presence of possible promoting factors. As things stand, it becomes almost impossible to predict a danger level, and the only advice that can be given with confidence is that every vestige of a carcinogen must be eliminated to insure safety.

The implications of the two stage mechanism in carcinogenesis are of immense importance in medico-legal considerations. Two recent publications have suggested that non-carcinogenic trauma combined with previous exposure to carcinogen may have been the cause of the emergence of tumors in two workmen[55,56]. Further case reports of this sort are, of course, necessary to lend weight to such contentions. However, when it is seen that trauma may exert an important action on the emergence of experimental tumors, it is clear that the evaluation of its role in occupational disease will become of importance.

In studies concerned with experimental animal tumors, whether induced, transplanted, or spontaneous, it is possible to observe the lesions from their inception to the death of the animal; in the case of the transplanted tumor, it is even possible to observe the progression of the tumor through a succession of

hosts. Certain investigators have been much struck by the variable life histories of tumors in animals when studied in this fashion, and in particular, in the lack of applicability of the rigid definitions of classical pathology. Although many studies have been devoted to the morphological description of experimental tumors, many of these have studied only the final appearance of the tumors; three studies in which particular emphasis has been given to the life history and changing pattern of the lesions will be considered in some detail. The studies of Rous and Kidd[31] of the so called "conditional neoplasms" induced by tar painting on the skin of the rabbit are of particular interest. These investigators tarred the ears of rabbits for some time and observed the pattern of tumor growth with great care; they additionally observed the effect of a cessation and subsequent resumption of treatment. They found that three types of tumors occurred initially, but would disappear if tarring were stopped, and would reappear in precisely the same location if treatment were begun again. These tumors were classified as papillomas, "frill horns," and carcinomatoids by these workers. The papillomas have previously been well described as stalked and broad based folliculo-adenomas by Yamigiwa and Itchikawa[25], who noted that the latter type were more vigorously growing, with carcinomatous change occurring in them quite often. Rous and Kidd found a similar type of lesion composed of stratified squamous epithelium with connective tissue cores that sometimes formed branches. They noted that these tumors could have a broad sessile base or could be stalked, but did not subdivide them from a terminological standpoint and merely called them papillomas. The second type of tumor they noted was the "frill horn," usually about 3 mm. across at the base, building up into a keratonized horn about 20 cm. in height, and largely restricted to the superficial layer of the epidermis. The third type of tumor noted was the "carcinomatoid" occurring rather early, having the appearance of a squamous cell carcinoma, including the characteristic of invasiveness. In eighteen rabbits tarred, a total of 129 tumors were observed of which fifty were clearly classified as papillomas, five as frill horns, and sixty-four as carcinomatoids; the remainder were called "transitional growths." With a cessation of treatment, a large proportion of the papillomas regressed as did the majority of the carcinomatoid tumors; the frill horns remained present. When tarring was resumed, the papillomas re-appeared in the same locations and some of them continued to grow progressively, eventually becoming truly malignant and metastasizing. The carcinomatoids, on the other hand, would regress even though tarring was continued, and some of them might eventually assume a papillomatous appearance. Even though some of the carcinomatoids were observed to have proliferated within the lymphatics, no instance of metastases was observed. It is perhaps interesting to compare the recently described kerato-acanthoma[57] of the human, that has the appearance of malignancy and yet can regress, with these tumors. The many questions that emerge from this study such as the truly neoplastic nature of such lesions and their general significance are well discussed by Rous and Kidd[58]. In the case of mouse skin tumors, certain differences have been observed, but the general pattern is the same[59]. 899 skin tumors arising in the skin of 100 mice, painted twice weekly with the carcinogen, 9,10-dimethyl-1, 2-benzanthracene as a 0.5% solution in mineral oil, have been studied in some detail. These tumors were classified again into three categories of benign tumor; sessile and pedunculated squamous cell papillomas were separated, and a third type described as a conical tumor. The sessile tumors were basically of two sorts; in one variety, the major portion of the tumor was composed of the fibrous core with minimal thickening of the epidermis, and but little hyperkeratosis; the second variety consisted mainly of epithelial tissue with considerable hyperkeratosis.

The pedunculated papillomas observed are similar to the stalked and broad based folliculo-adenoma, as described by others[25, 60]. The conical tumors, in addi-

TABLE I

ALTERNATIVE PATHS TAKEN BY SESSILE PAPILLOMATA

GROUP I (Tumors not progressing):	
Sessile papilloma throughout	631
GROUP II (Tumors progressing once):	
Sessile papilloma to pedunculated papilloma	102
Sessile papilloma to conical tumors	22
Sessile papilloma to carcinoma	66
GROUP III (Tumors progressing twice):	
Sessile papilloma to pedunculated papilloma to conical tumors	8
Sessile papilloma to pedunculated papilloma to carcinoma	32
Sessile papilloma to conical tumors to carcinoma	18
GROUP IV (Tumors progressing thrice):	
Sessile papilloma to pedunculated papilloma to conical tumors to carcinoma	4
Total sessile papillomata seen	883

tion to having the epithelial proliferation and hyperkeratisation, also have an epithelial crater extending deep into the dermis. These tumors are somewhat like a mixture of the frill horns and carcinomatoids of Rous, but do not, like the latter, regress. Of the 899 tumors studied, 560 were classified as sessile papillomas, 211 were peduculated papillomas, 28 were conical tumors and 100 were squamous cell carcinomas. Many of these tumors progressed through several of the observed types in the course of the experiment, and by maintaining weekly records on a gross morphological basis, the various possibilities were recorded.

Table I summarizes the large series of alternative pathways which may be taken by individual tumors.

Although the majority of carcinomas in this experiment occurred in pre-existing benign neoplasms, four carcinomas did apparently occur as such at their first appearance. A total of only eighteen tumors of the 899 seen regressed in this experiment, which is a very small number as compared with other experiments recorded[61].

In a fundamental study of the behaviour of spontaneous breast tumors occurring in BR hybrid mice, Foulds[62] concluded that tumors follow certain rules of progression. In this instance, as with the skin tumors described, the animals develop multiple lesions; grossly these tumors do not have the degree of morphological variation seen with the skin tumors, and Foulds studied growth rates and the effect on these tumors of repeated pregnancies. Pregnancy results in a stimulation of growth in some of these breast tumors, but not in others. It was possible, therefore, to classify these tumors immediately into responsive and unresponsive types; furthermore some tumors would respond by an increased growth persisting after the pregnancy and others would be influenced only temporarily, returning to their previous growth rate after parturition.

Defining "progression" as an irreversible qualitative change, Foulds proposed the following rules:

Rule I. Independent Progression of Multiple Tumours. Progression occurs independently in different tumours in the same animal.

Rule II. Independent Progression of Characters. Progression occurs independently in different characters in the same tumour.

Rule III. Progression is independent of Growth. Progressions occurs in latent

tumour cells and in tumours whose growth is arrested. Two notable corollaries of Rule III are: 1) At its first clinical manifestation a tumour may be at any stage of progression; 2) Progression is independent of the size or clinical duration of a tumour.

Rule IV. Progression is *continuous or discontinuous,* by gradual change or by abrupt steps.

Rule V. Progression follows one of *alternative paths of development.*

Rule VI. Progression does not always reach an end-point within the lifetime of the host.

From the studies of the skin tumors discussed previously, it can be noted that the rules of progression are followed extremely closely, and in all instances the conclusions reached by Foulds from studies with breast tumors are confirmed. Foulds has also studied a fibroadenoma of the breast of the rat and bladder tumors induced in mice with 2-acetylaminofluorene and concluded that the pattern of growth is the same as in these other instances[63].

In so far as the practical clinical implications of these studies are concerned, it is immediately obvious that many new studies are necessary to correlate these observations. In the case of mammary carcinoma in the human, it has been clear for some years that great variation exists from one tumor to the next. In studies by Nathanson[64], the fact that androgenic hormones will influence one series of mammary carcinoma, estrogens another series, and that many tumors are totally unresponsive has been well brought out, and used to form the basis of discussions on the intrinsic nature of tumors.

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CARCINOGENESIS AND METABOLISM OF CARCINOGENS*

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The role of the carcinogen in the intimate mechanism of carcinogenesis has been intensively investigated ever since Kennaway and Hieger[1] showed that an individual chemical, 1,2,5,6-dibenzanthracene (I), could be used to induce animal tumors experimentally. There is still almost complete ignorance as to the mechanism of carcinogenesis occurring at the site of contact of the carcinogen and the mouse skin. Underlying these studies has been the metabolism of the carcinogen.

While diverse types of carcinogens are known[2], interest has persisted with the carcinogenic hydrocarbons since they induce tumors at the site of exposure (*e.g.* mouse skin) relatively quickly. The possibility exists that the compound being painted is structurally close to the physiological carcinogen.

The classical approach of dosing animals with large amounts of the carcinogen and examining the excreta for metabolites has been extensively employed. Inherent to this method is the serious drawback that the excreted metabolites may be quite different from those occurring at the site of tissue response. The use of C^{14} labeled carcinogens combined with the carrier and tracer techniques surmounts this difficulty. This aspect of chemical carcinogenesis was initiated by C. Heidelberger[3].

The first requisite of the C^{14} tracer technique is a sample of the desired carcinogenic molecule with one or more of its C^{12} carbon atoms replaced by C^{14}, an isotope of carbon which is long lived (5,100 years half-life), and is a soft beta emitter (0.15 MEV). The synthesis of the C^{14} labeled carcinogen must start with $BaC^{14}O_3$ as this is the C^{14} material available from the Atomic Energy Commission. Thus even though the synthesis of the unlabeled carcinogen would be in the literature, a new total synthesis which starts with $BaC^{14}O_3$ is necessary to obtain the C^{14} labeled compound. Since the C^{14} atom must persist in the metabolites of the carcinogen, the position of the labeled atom in the present molecule must be selected to meet this additional requirement. Because of the above requirement only the metabolic studies can determine whether the C^{14} label has been incorporated in a suitable position of the parent molecule.

In order to identify a metabolite of the labeled compound the usual methods of identifying substances are of little use because of the minute amounts involved. The so-called carrier technique is used. In this method a non-labeled sample of the suspected metabolite, the carrier, is added in a measurable amount to an appropriate radioactive tissue fraction or extract and the carrier is isolated and purified extensively. If after repeated and exhaustive purification the radioactivity is maintained to an unalterable degree in the carrier, then the non-radioactive carrier and the source of radioactivity in the isolated carrier are identical and a metabolite has been identified. The carrier technique is not only sensitive but is also quantitative.

From the above modes of investigation it became apparent that the carcinogenic hydrocarbons are metabolised to phenols and quinones which retained the intact ring system and acids due to ring degradation. Because the metabolism of C^{14} labeled dibenzanthracene has been extensively investigated, the *in vivo* metabolism of this compound will be described in detail as representative of the carcinogenic hydrocarbons (see chart 1). The classical approach demonstrated that the phenolic compound, IV, was present in rat and mouse urine[4]. A monohydroxy derivative, VIII, with unknown orientation of the hydroxyl group, was found in rabbit feces[5], and IX, a dihydroxy derivative of unknown orientation of the hydroxyl groups, was found in rabbit excreta[6]. By means of the tracer

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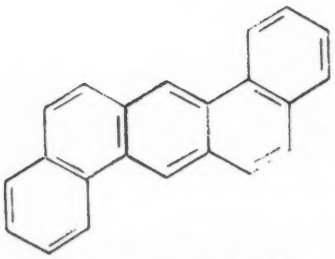
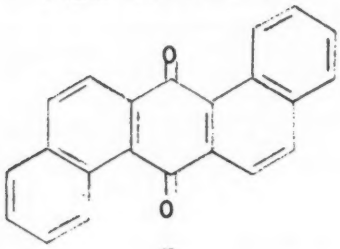


I 1,2,5,6—Dibenzanthracene



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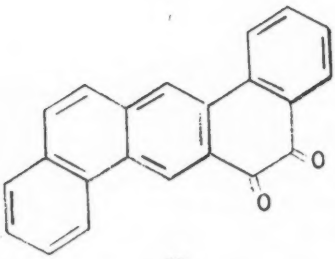

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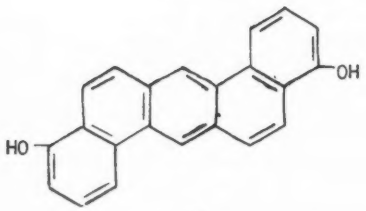

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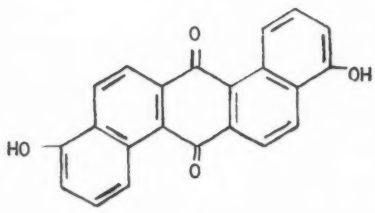

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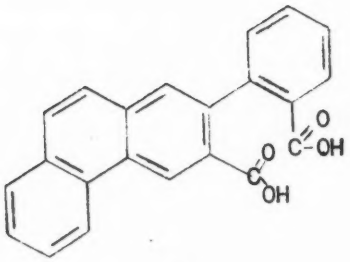

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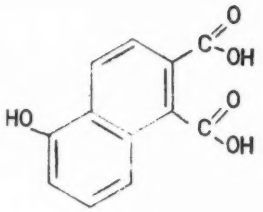

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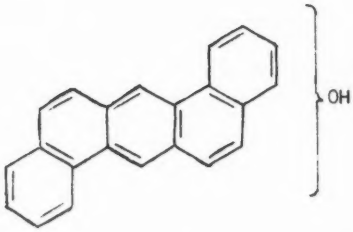

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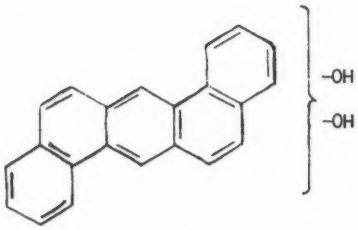

IX

technique, the quinone, II, was found in mouse skin and mouse liver[7], compounds III and IV only in mouse liver[7], and the dibasic acid, VI, in mouse skin[8] and the phenolic acid, VII, in mouse skin, liver and feces[9]. While metabolites detected in mouse liver, a tissue non-susceptible to the carcinogen, may be quite different from those in mouse skin, a susceptible tissue, the influence of intestinal flora has been eliminated and advantage taken of the extensive metabolic activity displayed by the liver. It is evident that primary biological oxidation has occurred in every ring of the original compound. This is of importance because previous to 1952 great emphasis and speculation was directed to the then current observation that the only metabolites isolated from dibenzanthracene or other carcinogenic hydrocarbons involved primary biological oxidation of the peripheral rings.

The nature of the compounds characterized in conjunction with the additional evidence that hydroxylation must precede ring cleavage[9] present two likely routes from I to VII. One sequence is I to II to V to VII, the other possibility is I to IV to V to VII. The evidence in hand does not permit a decision between the two pathways. None of the metabolites have been shown to be carcinogenic.

While the characterization of metabolites of a carcinogen is vital information, of much greater importance is the overall problem of the mechanism whereby the carcinogen acts. It is quite possible that the identified metabolites are due to detoxication and are irrelevant to the carcinogenic process.

An important turning point in the biochemistry of chemical carcinogenesis was the work of Miller and Miller[3], who, working with a different class of carcinogens, found that carcinogenic azo dyes were bound to liver protein (for an example of this class of carcinogen see reference 2). This observation took on significance when Miller and Miller correlated the level of bound dye with tissue and species specificity of carcinogenic and non-carcinogenic azo dyes. In the rat the only susceptible organ is the liver, and this is the only organ with protein bound carcinogenic dye. Non-carcinogenic azo dyes do not bind with liver protein. The livers of non-susceptible species, *e.g.* hamsters, chicks, guinea pigs and chipmunks, have no protein bound carcinogenic azo dye. The mouse which is somewhat more resistant than the rat has an intermediate level of dye bound to liver protein. Rat liver tumors, elicited by feeding the azo dye, have free azo dye, but no dye bound to its protein. Electrophoretic studies on the soluble liver protein showed that the bound dye was mainly combined with the slower moving *h* component. In the hepatoma, which has no bound dye, the amount of *h* protein component is markedly diminshed. Thus, the deletion hypothesis of chemical carcinogenesis was introduced, *i.e.* the carcinogen combined with a protein involved in growth control and this protein is not reproduced in successive cell generations.

An attempt was now made to extend the protein binding picture to the polycyclic hydrocarbons. The carcinogenic hydrocarbons, 1,2-benzanthracene[10], 1,2,5,6-dibenzanthracene[10,11,12], 3,4-benzpyrene[10,12,13,14], 9,10-dimethyl-1,2-benzanthracene[10,13,15], and methylcholanthrene[10], were found to be bound to mouse skin protein. However, the non-carcinogenic hydrocarbons 1,2,3,4-dibenzanthracene[10], 2′,6-dimethylbenzanthracene[14], and perylene[14], were also significantly bound to mouse skin protein. On the other hand, the non-carcinogenic hydrocarbons anthracene[13], and phenanthrene[13], were not bound significantly to mouse skin protein. Apparently there are deviations to the concept that protein binding of the compound studied is related to carcinogenic potentiality. Work in this laboratory has indicated that the level of binding with mouse skin protein not only depends on the nature of the carcinogen, but on the dose per unit area of skin and the increase in weight of the treated skin induced by the carcinogen[15]. Because of these two additional variables, the data in the literature correlating the level of protein binding and carcinogenicity is difficult to assess. However, it does appear that protein binding is a necessary biochemical aspect of carcino-

genesis, but is not in itself the unique portion of the carcinogenic process.

In view of the significance of protein binding, of utmost importance are the structures and physiological roles of the protein bound metabolite, the protein and the amino acids involved. The first such structural determinations has recently been reported[8]. The dibasic acid, VI, was shown to be bound to mouse skin protein subsequent to painting the mouse skin with C^{14} labeled dibenzanthracene, I. It is of further interest that biological degradation of the parent hydrocarbon had occurred at the site of the molecule called the K-region. Theoretical physical chemists[16] had predicted for many years that the K-region of a carcinogenic hydrocarbon was the portion of the molecule involved in the biochemical process resulting in carcinogenesis. The chemical description of the K-region is that individual double bond of the molecule which is most like the single double bond of ethylene, and least like the double bonds in benzene which is completely aromatic.

An intriguing observation in chemical carcinogenesis has been the variation of the response to the same carcinogen when the tissue and species are varied. This difference in the case of 2-naphthylamine has been traced to a metabolic difference. It was demonstrated[17] that the bladder tumors in the dog produced by feeding 2-naphthylamine are not caused by the parent compound, but rather by the metabolite 2-amino-1-naphthol. The dog which excretes fifty-five per cent to seventy per cent of the dose of the parent amine as the carcinogenic metabolite acquired bladder tumors, while the rabbit, rat, and mouse excrete much less of the carcinogenic metabolite and are hence less susceptible to the parent compound.

Urethane is another example of a carcinogen acting through a metabolic derivative[18]. Viable transplants of mouse fetal lung tissue which were exposed *in vitro* to urethane in Ringer's solution or rabbit serum failed to develop adenomas while adenomas of the lung tissue developed if the *in vitro* exposure had been to the serum of a rabbit which had been injected previously with urethane. Because of the simple structure of urethane the determination of the structure of the intermediary compound may result in a new concept concerning the nature of a contact carcinogen.

This survey is notably incomplete because of the omission of the well documented description of the metabolism of the carcinogenic azo dyes and the systemic carcinogen 2-acetylaminofluorene, which incidentally also binds the protein of rat liver[19]. It is uncertain how far the above approaches will go in elucidating the mechanism of local carcinogenesis, especially if the agent induces carcinogenesis in a relatively small number of cells. If this be true, the present analytical methods, even the C^{14} tracer technique, cannot detect the unique biochemical interaction leading to carcinogenesis. The prime problem is still to prove experimentally that the metabolism being investigated is related to the carcinogenic process.

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THE CARCINOGENIC POTENTIALITY OF AIR POLUTION*

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Epidemiologic studies have demonstrated a marked and significant increase in the incidence of cancer of the lung in the last decade for which a variety of explanations have been suggested. This increase is considered by most authorities to be a real phenomenon, not only the apparent result of improvement in diagnostic methods or of the progressive ageing of the population[44, 46, 107]. In order to understand the role played by various factors responsible for this phenomenon, it is necessary to examine some of its aspects in detail.

The increase in cancer of the lung began about 1914 and became more and more evident during the 1930 period[21, 23, 83, 107]. The statistics derived from death certificates, as well as those obtained from autopsy material, show a similar increase in all industrialized countries, as can be seen from data reported in Denmark, Italy, Germany, England, Norway, United States, and Switzerland[46, 57, 91]. Various detailed studies of cancer of the lung in England and Wales show an increase in incidence estimated at approximately nine times between 1928 and 1947[18, 44, 55, 56, 107, 108]. The figures become quite different when we consider the incidence for the male and the female population separately. The proportion between them is highly variable, between 2:1 and 20:1. The prevalence is always in the male sex and has particularly increased in the last few years[45]. In 1914, both the level and the shape of the curves for death by age were essentially the same for males and females in the United States. In the following thirty-five years, the shape of the mortality curve for males changed radically, while that for females remained relatively unchanged[25]. As far as age groups are concerned, the mortality rates for cancer of the lung in a cross section of the male population for a given year, show a peak of maximal incidence at seventy years of age and then a decrease. However, when the same figures are observed for the groups of population born in the same year, called cohorts, and then followed up, the mortality rates are constantly increasing.

According to Dorn[24], this data would be consistent with the supposition that some new agent capable of causing cancer of the lung has become operative during the past half-century; that this agent has affected males more strongly than females, and young adults earlier than older adults. Another consideration can be advanced when one analyzes data which shows clearly that the death rate for lung cancer is higher in urban than in rural areas. In a survey of lung cancer death rates by states for 1948, the maximum was reached in New York (11.9 per hundred thousand) and the minimum in Idaho (2.9 per hundred thousand)[49]. Curwen, Kennaway and Kennaway concluded in a recent study that the standard mortality rate for lung cancer in both sexes increases with the increase of urbanization[18].

In the evaluation of increase in lung cancer, some possible causes of statistical error should be kept in mind. The introduction of antibiotics, the attention directed towards the diagnosis of cancer, the improvement in diagnostic methods, and the more efficient collection of epidemiologic data, all have to be considered in attempting to evaluate the validity of these statistics. No study has yet been undertaken to establish the real importance of these factors. Superficially, however, they do seem to be insufficient in explaining the characteristics of distribution of this observed increase. A change in genetic susceptibility cannot be accepted because it cannot manifest itself within the life span of one generation.

At this point, it is impossible to avoid a discussion, even briefly, of the importance of tobacco smoking, particularly

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cigarette smoking in the pathogenesis of lung cancer. This possible etiological factor was repeatedly suggested in the past[68, 72, 92], but it was only brought to the attention of the public in the last five years following the publication of results of several statistical and experimental studies on the subject[21, 22, 43, 65, 71, 74, 94, 116]. It is impossible to analyze the details of this material. We can only observe that there are considerable differences between the results of various authors. Moreover, while the increase in lung cancer is higher in the male population, the increase in cigarette consumption in the last two decades is at least as high in the female population. Again, the parallelism and synchronism between the curves for cigarette consumption and the incidence of lung cancer do not justify a cause and effect relationship because they do not take into account the necessary latent period. In a very recent criticism of the statistical methods used to prove the association between smoking and lung cancer, Berkson[9] indicates the unreliability of certain apparent associations. Considering the statistics of Hammond and Horn, collected by the American Cancer Society[43] by means of distribution of a questionnaire, Berkson observes that the male population considered is a biased sample because, compared with the general male population of the United States, it contains a higher percentage of non-smokers. The death rates of the surveyed populations are much lower than they should be when compared with the general population of the United States. If the group of non-smokers is then considered, it is seen that their death rate is lower than that of the general population; the non-smokers of the American Cancer Society, comments Berkson, must be a group of "phenomenally hardy men". It must be assumed that people in good health show a tendency to exclude themselves from investigations. The cigarette smokers in the population used by the American Cancer Society have a death rate higher than non-smokers for cancer of the lung, but they also have a higher death rate for other forms of cancer, coronary artery, and other diseases. If the statistical association between cigarette smoking and lung cancer has to be considered as proof of a causal relationship, then a similar relationship must be said to have been demonstrated between cigarette smoking and the many other diseases which these same statistics link. The question that one must ask as a result of this survey is not "Is cigarette smoking the cause of lung cancer?" but "What is not caused by cigarette smoking?" Continuing the comparison between the data of this selected population and those collected for the entire male white population of the United States, it can be observed that the smokers of the American Cancer Society have a lung cancer death rate lower than that of the general population[9]. These observations indicate the difficulty in collecting reliable data where any form of selection may be present in the groups of population to be compared.

The mortality rates for lung cancer collected for eight cities in 1947 by the biometric section of the National Cancer Institute show that the highest difference is between the cities of New Orleans (39.1 per 100,000 people), and that of Atlanta (13.4 per 100,000 people). These two cities are both situated in the same part of the United States and, therefore, it is difficult to attribute this difference to varying smoking habits[49]. Another observation can be made from the same statistics when data collected in 1937 is compared with that collected in 1947: the percentage increase in mortality for this decade is maximum for Dallas (455 per cent), an area with considerable recent industrialization, while, it is minimal for Chicago (105 per cent), a city that has been industrialized for decades.

These observations indicate a close relationship between the increase in lung cancer and environmental factors, particularly with industrialization. As observed by Hueper[48], all environmental agents, recognized or suggested as possible causes of lung cancer, are inhaled. But atmospheric contaminants may also gain entry by skin contact or by ingestion. Through the atmosphere, water, food, and soil can be contaminated. This is important because increasing industrialization has wrought fundamental changes in our environment. This is par-

ticularly true of the chemical composition of the air we breathe. An ever increasing number of new chemical substances are introduced in great quantities into the atmosphere, many of which are, or are suspected of being, carcinogenic agents. Therefore, a better descriptive and analytical knowledge of the composition of the atmosphere is needed if we are to interpret properly the relationship between air pollution and carcinogenesis.

Knowledge of the mechanism of tumor production in the lung is still to be desired. Many factors, and their inter-relationship, have to be considered. In this laboratory, we are studying with the aid of radioactive materials the ability of carcinogenic hydrocarbons to penetrate the respiratory epithelium, and the mechanism of their combination with cellular constituents of the lung.

The methodology for the study of the carcinogenic effects of air pollution must be founded on three fundamental points: 1) the analysis of the chemical composition of air pollution in general, particularly in special conditions arising from different industrial operations; 2) the study of the biologic effects of the contaminants on laboratory animals; 3) the collection and evaluation of epidemiologic data concerning populations and groups exposed to particular atmospheric pollutants.

In the study of the chemical aspects of air pollution, it is essential to identify even the smallest concentration of any material present in the air. Some of these have already been recognized or suspected as carcinogenic as early as 1920 (Passey, Campbell, Kennaway, and others)[12, 13, 14, 53, 54, 80, 81]. These authors were able to induce tumors by treating animals with the combustion products of wood, charcoal, pit coal, and shale oil. Powders derived from tarred roads containing two to three per cent tar were shown to be carcinogenic, as were different soots[86]. In 1942, Leiter and Shear[66], and subsequently together with Shimkin[67], obtained malignant tumors in the mouse by subcutaneous injection of tars extracted from dusts of urban atmospheres. In the last decade, catalytic cracking of petroleum was introduced, and it has been shown by Smith and his co-workers[105] that the fractions with a boiling point above 700°F. are carcinogenic. Subsequently, promoting activities have been shown for fractions with a lower boiling point[103, 104, 105].

The problem of the chemical nature of the substances responsible for these activities began to be clarified by the work of Kennaway and Cook which led to the identification of 3,4-benzpyrene as the carcinogenic agent in coal tar[16]. Afterwards, a large number of other aromatic polycyclic carcinogenic hydrocarbons were identified and synthesized. Benzpyrene is now known to be but one of the carcinogenic hydrocarbons present in tar and shale oil[7, 8]. Its presence has been demonstrated in domestic soots[38], exhaust gases of gasoline and diesel engines[62, 63, 113], and particles produced by the friction of automobile tires[31, 32, 33]. Benzpyrene is an important substance to consider in the chemical analysis of the atmosphere, but other hydrocarbons are also emitted from the same sources of pollution. There is no proof that benzpyrene has a carcinogenic action in man. Therefore, to use the concentration of benzpyrene in the air as a measure of man's hazard is to be discouraged. Many other aromatic polycyclic hydrocarbons may also contaminate the air: some have been identified chemically, others only spectrographically[61]. Needless to say, a great deal of analytical work remains to be done.

The importance of this work is revealed in the fact that the hydrocarbons emitted in the Los Angeles atmosphere in 1951 reached an average of 1932 tons a day, of which 1866 tons were derived from petroleum[39, 40]. Studying the chemical composition of the Los Angeles atmosphere, Haagen-Smit showed in 1952 the importance of oxidizing mechanisms that may act on the easily oxidizable materials derived from petroleum. He concluded that for an exact evaluation of the effects of a polluted atmosphere, changes occurring in the atmosphere must be considered, as well as the nature of the original emission[99]. Therefore, substances that are not initially dangerous, may become dangerous by the

action of other components in the same atmosphere. A new course of carcinogenic material has been suggested by Kotin[61] as a result of this oxidizing power of polluted air: during the oxidation of hydrocarbons, he suspected the possible formation of epoxides and diepoxides, compounds which may be a new class of atmospheric carcinogenic agents[59].

Compounds of the aliphatic series have recently been of considerable interest, both for theoretical and practical purposes. Experiments indicate carcinogenic activity of an aliphatic fraction derived from a polluted atmosphere; this fraction consists of oxidation products of automobile gasoline obtained with ozone and light energy[60]. This work. however, cannot be considered conclusive owing to the difficulty in excluding the presence of aromatic contaminants. Alpha and beta naphylamine and benzidine are compounds well known for their carcinogenic effect on the urinary tract of man and animals; they may all occur in certain polluted atmospheres as a result of waste disposal procedures.

Another important group of carcinogenic air pollutants are certain metals; some of which have been successfully used to induce tumors, others being incriminated on the basis of statistical data. Vorwald used beryllium to induce lung carcinomas in the rat[111]. Plastics and asbestos have also been incriminated[22, 77, 78]. Among the plastics suspected of being capable of inducing neoplastic changes one can list cellophane, polyethylene, polyvinyl chloride, tetrafluorethalene, polymerized teflon, polysilicone (silastic), polyamide, polyaceclate, polysterene, and bakelite[27, 28, 34, 75, 76, 77, 78, 79, 110, 117]. However, opinions are split on the significance of the epidemiologic data concerning the carcinogenicity of asbestos[46, 49, 91]. These substances are mostly the contaminants of limited areas and may act only on groups of people employed in special work. The rapidity of industrial expansion and the increasing use of natural and synthetic polymers indicate the importance of accurate observation in this field in the future[46].

The last category of compounds that must receive the attention of the chemist studying air pollution are radioactive substances. Their carcinogenicity is widely proved, both epidemiologically and experimentally. It is interesting to observe that one of the two experimental examples of the induction of bronchial carcinoma in the rat has been obtained by Lisco and Finkel[69] by the inhalation of the radioactive element, CE 144.

The second group of studies is concerned with the biologic effects of air pollution on laboratory animals. In this type of study, the problem of experimental method is of great importance. Precise quantitation of animal tumor induction experiments in terms of human dosage ranges and even modes of exposure and entry have been difficult. Many investigators have been forced into the somewhat drastic position that any substance found to be carcinogenic in the laboratory, by whatever route it is administered and at any dosage level, should be considered as a potential carcinogenic hazard to man. One of the most difficult aspects of such investigations concerns the difficulty in proving a suspected agent innocuous; certain recent studies demonstrate how difficult this may be[84, 103].

The significance of the results of experimental carcinogenesis in human pathology has been well described by Steiner[106] who points to the many similarities in the two fields: in the morphology of tumors, the nature and the specificity of carcinogenic agents, the latent period for the induction of tumors, the relationship between dose and effect, and the effect of the summation of doses. The latter is particularly interesting. The pharmacologic action of carcinogens is irreversible, and repeated doses of carcinogenic substances, however small, over long intervals, act with a summation effect as shown by the studies of Druckrey[26]. The latter showed the dependence of the carcinogenic effect on the summation of partial doses independent of concentration. There is no minimal level of concentration, therefore, that can be considered harmless provided that the frequency of exposure is enough to induce by summation a total dosage that

is carcinogenic. Experimentally, minimal quantities of carcinogenic hydrocarbons have been shown to induce tumors: as little as 0.0004 mgm. of 1,2,5,6-dibenzanthracene can induce sarcomas by subcutaneous injection[96].

A single, minimal dose of a carcinogenic substance, although unable by itself to induce the appearance of a tumor, may be able to induce a cellular change—a latent tumor change—that is irreversible. Other factors, even non-carcinogens, may act subsequently to induce the rapid growth of tumors. These factors are called promoting agents or co-carcinogens. We know very little at the present time about the synergism and activity of different carcinogens. However, various experimental results seem to indicate the existence of such mechanisms[2, 37, 17], either the direct result of a combined action on an organ[10, 42, 84], or mediated through circulating metabolites[85].

Investigations of the human hazard of different types of air pollution must be concluded with a study of the epidemiologic data. In this field, the study of the carcinogenic effects of air pollution must be separated into two distinct types. The first concerns the effects on large groups of population of contaminants that enter the general atmosphere, while the second deals with the pollutants emitted by industrial areas. Therefore, the latter usually involves only limited, easily identifiable groups of people. The epidemiologic and prophylactic measures that have to be faced are quite different.

Two systems of epidemiologic inquiry may be used to establish the responsible factors for carcinogenic action. The "prospective" method consists of the collection of complete data for a given population, exposed to given environmental or occupational conditions in a given period of time. By this approach, the history of each individual is investigated to establish the incidence of the type of tumor under study. The "retrospective" method involves the detection of all tumors in a given population through specialized clinical centers where precise information is collected concerning the environmental or occupational exposures of the patient in order to determine a significant type of exposure. The results of such statistical studies must be evaluated with great caution. We have to give the greatest attention to the statistical reliability of the data, to the reliability of controls, to the selection of subjects, and to all other possibilities of error.

At the present moment, the epidemiologic data available concerning the formation of tumors by exposure to polluted atmospheres is extremely debatable. It is highly desirable that in the near future our present data be extended and widely documented. Our epidemiologic knowledge of the pathogenicity of coal and petroleum by inhalation of combustion and distillation products is very scarce and equivocal[46]. The most indicative observations are those of Kawahata[51, 52] on gas generator workers in a Japanese steel mill with an incidence of lung cancer of five per 1000, and more recently of Cruickshank[17] and Doll[20] concerning the carcinogenicity of shale oil and petroleum derivatives which are inhaled[49]. The manufacture of isopropanol has been associated epidemiologically with a high incidence of carcinomas of the paranasal sinuses, lung tumors, and tumors of the vocal cords[114]. There are very few statistical observations on groups exposed to the inhalation of various inorganic substances. Cases of cancer of the nasal cavity, paranasal sinuses, and of the lung have been collected in nickel workers[118] and a significant incidence of lung cancer has been observed in chromate workers[1, 46, 49]. Other observations in the German mines at Schneeberg and Joachimsthal attribute a high incidence of lung cancer to radioactive substances.

The investigation of chemical and physical agents have been always important in the study of the etiology of tumors. However, it is only recently that we have come to realize the importance of air pollution in carcinogenesis. We are now in a situation not too different from that of the bacteriologists at the beginning of the twentieth century who were faced with the problem of assimilating and classifying many seemingly diverse facts. Many carcinogens have been described

and some are known to be in the atmosphere; others will be discovered in the future. The pathophysiology of neoplasia is still not understood. Still less is known about the effects of air pollution. Accurate investigation into the constituents of our atmosphere, together with a study of contaminants and their biologic action, thus imposes itself as a problem which must be conquered.

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QUESTIONS AND PROBLEMS OF PSYCHOPHARMACOLOGY*

ERMINIO COSTA, M.D.**

For many years the pharmacologic therapy of psychotic behavior has been limited to the use of barbiturates and other sedatives with very limited success. Recently, however, a new experimental approach to the problem has been made available by the discovery of a series of compounds which have marked psychogenic effects. Among these is D-lysergic acid diethylamide (LSD)[2, 4, 11, 46, 53-55], a semi-synthetic derivative of ergot. Changes in behavior due to ergot poisoning have been known for a long time; however, intensive study of the psychogenic effects of LSD did not start until its accidental discovery by Hoffmann. This investigator, while studying the oxytocic activity of a series of semi-synthetic alkaloids chemically related to lysergic acid, inhaled some of the material and noticed marked changes in behavior[53, 54]. In common with other lysergic acid derivatives, LSD has oxytocic[52] and other effects, but in addition, it has a characteristic action on the central nervous system[7]. In the guinea pig LSD induces motor excitement during narcosis; in the dog it causes autonomic reactions and alterations of behavior; in the monkey these changes of behavior are more marked and, probably, blindness is also present. In normal men an oral dose of fifty to one hundred gamma causes a loss in precision of intentional movements, faulty articulation of speech, incoordination, clonic spasms, euphoria and manic behavior (or, less often, hebephrenic-like apathy), aggressiveness, indifference, and occasionally, suicidal ideas. In addition, hallucinations and changes in visual, auditive, taste and smell functions have been described. Similar reversible psychoses can be produced by the administration of mescaline, the active principle of "peyote"[23]. Peyote is a root used by certain Indian tribes during religious ceremonies for the purpose of obtaining a mystic exhaltation, believed to be due to a sort of communion with a deity present in the root itself. Although mescaline and LSD are historically unrelated and are derived from botanically unrelated plants, they are related chemically, both being derivatives of indole. Indeed, similar changes in behavior can be produced by the administration of numerous other indole derivatives[59], such as by introducing serotonin (5, hydroxytryptamine) into the lateral ventricle of the cat[15], or administering bufotenine (N, N, dimethyl-5, hydroxytryptamine) to monkeys[13]. Injecting serotonin intravenously into humans, produces dizziness and other peculiar sensations in the head[35, 51]. The mechanism of action of these drugs, the production and control of experimental psychosis and their value in clinical medicine are now under active investigation in many laboratories where many biochemical and metabolic changes have been observed[3, 8, 16, 27, 28, 31-33, 37]. For the most part, the physiological meaning of these changes and their practical importance are still obscure, as one of the biggest difficulties in the solution of these problems is the lack of sensitive and objective methods for evaluating the effects of the drugs. Among the methods now available for psychopharmacological investigations, electroencephalography is the most useful. Electrical manifestations originate at the level of the nerve cell; they depend upon cellular activity and can be recorded, by means of special electrodes, either from single neurons or from large cellular aggregates at various levels of the central nervous system. The electroenecephalogram (EEG) ob-

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tained in this manner, represents the algebraic sum of all electric events taking place in the cell bodies, dendrites, axones, and synapses in the area which the electrodes are recording, and it takes the form of a sequence of waves which differ according to the different areas of the central nervous system and to their functional status. Extreme changes in physiologic activity of various regions of the brain may be detected either by the appearance of hyperactive waves (the so-called convulsive pattern), or by a decrease in electric activity (the so-called pattern of rest[12]). Between these two types, there lies a whole system of functional patterns, the significance of which is not fully understood. Sleep and awakeness are considered expressions of opposite extremes of brain function and also give rise to characteristic EEG patterns which alternate variably between these two physiologic states. This alternation, which according to Magoun[29, 34], is controlled by the mesodiencephalic activating system (brain stem reticular formation and its anatomical projections to all parts of the cortex[29]), can be modified by means of drugs, so that either one or the other of the two typical patterns may be made to appear and persist[42]. For example, cholinergic drugs cause the appearance of the pattern of wakefulness (alert pattern), while atropine corrects the alertness induced by parasympathetic drugs and causes the appearance of the sleep pattern[40]. These phenomena are observed in the intact animal, but not in the isolated brain hemisphere preparation[41], lending support to the hypothesis that the site of action of atropine and of cholinergic drugs is the reticular formation, a structure which appears to act as a harmonizer of various brain functions. The action of atropine on the reticular formation may be related to its therapeutic effects in Parkinsonism[17, 25, 36, 38, 57]. It appears to be independent of peripheral anticholinergic effects, since other parasympatholytic drugs pharmacologically related to atropine, do not show proportional anti-Parkinson activity[42]; still others like congentin, (tropine benzohydril ether-methane-sulfonate), which is chemically related to atropine, are more active than atropine both as a sedative of the reticular formation and as an anti-Parkinson drug[44].

Characteristic EEG patterns can be evoked also by the administration of substances which are believed to be responsible for the chemical transmission of the nervous impulse in the central nervous system. Among these substances are acetylcholine and, according to Marrazzi, epinephrine and serotonin[30]. The EEG patterns evoked by cholinergic and adrenergic drugs are similar, but can be differentiated; for instance, the transection of the brain stem at the level of the inferior collicula prevents the EEG effect of adrenergic, but not that of cholinergic drugs. Dibenamine blocks the pressor effect of epinephrine but does not prevent the appearance of the alert EEG pattern, while atropine antagonizes the CNS stimulating activity of epinephrine, without modifying its pressor effects[45].

The interpretation of these findings is complicated further by the fact that the so-called EEG "sleep pattern" does not necessarily coincide with sleep. The atropinized animal, for instance, exhibits a "sleep pattern" whether he is asleep or awake[58]. Sleep and alertness are expression of opposite functional states of the central nervous system, but unfortunately, we do not know how closely they are correlated with the so-called "sleep" and "alert" EEG pattern, and while physiologic sleep is characterized by a "sleep" pattern, the presence of this pattern does not necessarily indicate that the animal is asleep. Similarly, the hypnotic effect induced by means of drugs (*e.g.* barbiturates) may not correspond to a depression of electrical activity in the reticular formation, which may appear sometime later[39], while the sedation induced by analgesics (morphine) may occur without any signs of a decreased electrical activity[58]. These are but a few examples illustrating the difficulties which one encounters in attempting to evaluate the meaning of electrical phenomena in the brain as biological bases for mentation: the fine

coordination of cerebral functions defies oversimplified explanations.

The so-called hallucinogenic drugs, mescaline and LSD, also have marked effects on the EEG. Although the precise interpretation of these effects must be left to future investigators, we may describe them and suggest their possible meaning and clinical importance. Mescaline in any amount[43] and LSD in small doses[5] cause the appearance of the alert pattern, while LSD in large doses causes the appearance of a resting pattern[43]. The psychogenic and electroencephalographic effects of small doses of LSD are blocked by frenquel (4, piperidyl-diphenyl-carbinol hydrochloride)[14,43], one of the so-called tranquillizing drugs. This is a very interesting pharmacologic phenomenon since frenquel does not modify the alert pattern induced by amphetamine or by cholinergic drugs[43]. Like frenquel, chlorpromazine and reserpine, which also are used as tranquillizing drugs in the treatment of psychotic patients, modify the EEG, although in different ways: reserpine always causes alertness[39], while chlorpromazine may cause depression (in small doses) or alertness (in high doses)[22]. The clinical indications of these drugs are also different: chlorpromazine is the drug of choice in the treatment of patients with paranoid and schizoaffective reactions; reserpine is the drug of choice in the treatment of hebephrenic and hebephrenocatatonic patients, and frenquel is used in the treatment of the hallucinatory and delusional features of schizophrenia[22].

Very little is known about the relationship between chemical structure and pharmacologic or therapeutic activity of tranquillizing agents. The demethylation of reserpine does not appear to modify its activity[48], while the introduction of a chlorobenzene radical into the side chain of chlorpromazine causes some potentiation of its tranquillizing activity[26]. The chlorine in the phenothiazine nucleus seems to be of some importance, as no phenothiazine derivitives more active than the chlorinated ones have been found as yet. The introduction of chlorine into the side chain increases the therapeutic activity of the drug, but also its toxicity: the new product (NP207), for example, can cause retinitis[26], an effect never observed with chlorpromazine.

Mescaline[10], LSD[6,18,21], and tranquillizing drugs[10] interfere with certain biological reactions mediated by serotonin, a substance found in acetone extracts of animal brain[1,56] and related chemically to those indole derivatives[20] which are endowed with psychogenic activity[59]. The antagonism between LSD and serotonin has been studied principally using the isolated uterus of spayed rats (which contain specific serotonin receptors)[19], the lungs of the guinea pig *in vivo*[21], or certain spinal reflexes in the dog[49]. The results of these studies suggest that the psychogenic action of LSD is due to inhibition of serotinin activity in the brain, although more recent studies have revealed that small doses of LSD (0.1 gamma/liter) facilitate the action of serotonin on the rat uterus[10] and on the spinal reflexes of the dog[49], rather than inhibiting it. This dual effect of LSD may offer a possible explanation for the dual effect that this drug has on the EEG, causing the appearance of the alert EEG pattern in small doses and of the sleep pattern in large doses[43]. If the relationship between the psychogenic effect of LSD and serotonin were confirmed, it may offer an explanation for the observation that human beings chronically treated with LSD develop a resistance to its psychogenic effect rather quickly[9,24] as if, by repeated administrations, large inhibiting concentrations of the drug were reached. This resistance disappears when the drug is withdrawn and its concentration in the brain is again lowered to values where serotonin facilitation is possible. In agreement with this hypothesis, mescaline which does not antagonize serotonin in the rat uterus, but rather displays a certain afnity for serotonin receptors, activates the EEG at all dose levels. No resistance to its effects appears to develop. This hypothesis may explain the therapeutic results which have been obtained with LSD in mental diseases[47,50]. The hypo-

thesis that the hallucinogenic effects of LSD are due to serotonin antagonism is weakened by the following observations: 1) many compounds, including some chemically related to LSD, antagonize serotonin activity in the rat uterus, but have no psychogenic effect[7]; 2) serotonin and LSD are not always antagonists: for example, the inhibiting effect of LSD on electrically activated respiration and glycolysis of guinea pig's brain *in vitro* is not inhibited, but enhanced by serotonin[27]; 3) LSD and mescaline do not antagonize serotonin in certain brain preparations[30]; 4) serotonin, hopefully administered in schizophrenic patients on the basis of this hypothesis[60], had no beneficial effects, suggesting that serotonin deficiency is not a factor in the pathogenesis of mental diseases. If it were possible to apply to the brain the results obtained on the uterine musculature, one could suggest the alternate hypothesis that tranquillizing effects are due to serotonin antagonism, while hallucinogenic effects depend upon serotonin facilitation.

Although much work remains to be done to elucidate the possible significance of serotonin in normal and abnormal mental functions of man and to evaluate the pharmacologic and therapeutic value of the so-called tranquillizing drugs, the basis for a pharmacological attack on the clinical problem of psychosis has been laid.

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CLINICOPATHOLOGIC CONFERENCE

Presented at Mount Sinai Hospital, Chicago, Illinois

DR. L. FELDMAN, Chairman **DR. H. RAPPAPORT, Secretary**

CLINICAL HISTORY

Initial Admission: A sixty-four year old man was admitted to Mount Sinai Hospital on August 2, 1954 complaining of unusual tiredness and lethargy. For the previous three weeks he had felt exhausted and his vision was becoming progressively poorer. One week before admission he was examined by an ophthalmologist. The patient had always been in good health until the present illness, but his wife stated that he had been a heavy drinker for the past thirty years. She said he was regarded as a peculiar person, who was rather "unsocial" and worriesome. Systemic review and family history were non-contributory.

Physical Examination revealed an acutely ill individual who appeared to have difficulty understanding what was wanted of him and who was very slow in answering questions. Perseveration was noted in his responses. Pulse rate was 80 per minute, temperature 98.0°F., respiration was 20 per minute, blood pressure was 220/110. Examination of the head revealed no abnormalities. The heart was enlarged slightly to the left but there were no other abnormalities. The abdomen was normal. His mental status was described as that of a mild stupor with confusion. There was a motor aphasia and right sided hemiparesis. Oppenheim's sign was positive on the right. Biceps, triceps and patellar reflexes were hyperactive on the right. Eye examination revealed right homonymous hemianopsia, arteriosclerotic changes of the fundi, and obliteration of the disc margins suggesting early papilledema.

Spinal tap on the day of admission revealed a clear fluid, with an opening pressure of 260 and a closing pressure of 240. The Queckenstedt maneuver was negative.

A tentative diagnosis of left frontotemporal neoplasm was made and a neurosurgical consultation was called. Over the subsequent four days the condition of the patient rapidly became worse. Complete right hemiplegia developed and he became completely aphasic. Four days after admission an exploratory operation of the left parietal region was done. The dura mater was tense; small fragments of the cortex were taken for histologic examination, which was reported as showing acute necrosis, acute inflammation, and reactive gliosis, etiology unknown. After the operation there were days when he was somewhat alert and could respond to simple commands; he remained aphasic, and the only movements he could make on command were slight movements of the left arm and leg, head, and tongue. The right side remained completely hemiplegic. A urinary infection which complicated the postoperative course was treated successfully. On September 23, the patient was discharged to a convalescent home, after a hospital stay of 7 weeks and 3 days.

Final Admission: The patient was readmitted to the Mount Sinai Hospital on October 1, 1954, one week following discharge. Aphasia and right hemiplegia had persisted.

Physical Examination revealed the patient to be stuporous. He was able to move his left arm and leg slightly on command, but did not respond at all to questioning. There was right facial paralysis and right hemiplegia, with the right leg held spastically in extension and the right arm in flexion. Babinski's reflex was present on the right side. Rossolimo's and Mendel-Bechterew reflexes were absent. Examination of the lungs disclosed many crepitant rales over both bases. There was a healing left craniotomy scar of the previous operation. Blood pressure was 170/110.

The patient's condition became progressively worse and he expired on October 12, 1954, twelve days after the final admission and ten weeks after initial admission.

LABORATORY DATA

Examination of the blood on admission showed a red blood cell count of 5.09 million, a hemoglobin of 16.9 grams per cent, or 108 per cent, a color index of 1.05, a white cell count of 12,050, with seventy-seven per cent segmented neutrophils, two eosinophils, one basophil, sixteen lymphocytes, and four monocytes.

Urinalysis on admission revealed a pH of 6.0, a specific gravity of 1.026, negative for protein, sugar, acetone, casts, red blood cells, and white blood cells. Subsequent urinalyses showed on August 6, 1954 a three plus test for protein, an occasional granular cast, and "loaded" with white blood cells. The findings returned to normal until August 30, 1954 when a trace test was obtained for protein, rare casts were seen, and eighteen to twenty-five red blood cells and thirty to forty white blood cells were noted per high power field. A urine culture was positive for E. coli.

Serum glucose was 118 mg., serum urea nitrogen was 15.0 mg., total protein was 8.10 gm., true albumin 5.10 gm., total globulin 3.0 gm., with a true A-G ratio of 1.70, thymol turbidity of 1.2 units, acid phosphatase of 0.17 Bodansky units, alkaline phosphatase of 4.67 Bodansky units, serum cholesterol of 287 mg., cholesterol esters 67 per cent, and a negative cephalin-cholesterol flocculation after twenty-four hours.

Spinal fluid examination on admission showed a protein of 57 mg., a true cerebral spinal fluid glucose of 45 mg., chlorides of 695 mg., a trace Pandy test, and no cells. The Kahn and Kline tests were negative.

CLINICAL DISCUSSION

*Dr. I. Finkelman**: This is an interesting diagnostic problem because the patient had been an alcoholic for the previous thirty years, causing personality deterioration, and confusing the clinical picture. For a few weeks prior to his admission he felt weak, was lethargic, and lacked spontaneity. The onset was gradual, without loss of motion, sensation, or consciousness. The gradual onset was against a vascular lesion and in favor of a cerebral neoplasm. On admission the only pathological reflex present was an Oppenheim reflex. There was mild papilledema. On the next day, there was weakness on the right side and he lapsed into an arousable stupor. That is more frequently found in brain tumor patients than early deep coma. He became aphasic. The gradual onset with right hemiplegia, aphasia and papilledema indicated a cerebral neoplasm and surgery was indicated.

Following the operation, the patient became alert due to the decompression, the lowering of the intracranial pressure. He improved and was taken to Rest Haven Convalescent Home. However, he became progressively worse and was readmitted to the hospital. He finally expired. The biopsy specimen was examined and no evidence of tumor was found; however, the clinical history was typical of a tumor, and the biopsy diagnosis did not give the true picture since tumor tissue had not been sent to the pathologist.

*Dr. J. Arendt***: A space-occupying lesion in the brain is evident roentgenologically only if it either displaces a fixed structure, such as the pineal gland; exerts pressure on bone, particularly such a delicate structure as the sella tursica; or if the lesion contains sufficient calcium to become conspicuous, as glioma, astrocytoma, craniopharyngioma, or meningioma sometimes do. Otherwise, contrast has to be created by air inflation as in encephalography, ventriculography, or, as an alternative method, by vascular injection in cerebral angiography.

We see in this case (Fig. 1) the skull of an old individual with senile osteoporosis, which actually is an age atrophy. The sella tursica is of normal width and configuration. The pineal gland is calcified and shows no displacement according to the tabulation of Vastine. The vascularity of venous markings is increased over the frontal bone but poor over the

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Figure 1. Lateral view of skull showing senile osteoporosis, normal sella tursica, and no displacement of the pineal gland.

parietal bone. There is normally such great variation of vascular markings that this sign has no great significance without accompanying bone changes. We have, then, no roentgenological evidence from these films to supply information of any brain pathology, but this does not rule out a tumor of the brain which Dr. Finkleman so decidedly and convincingly diagnosed.

*Dr. G. Coe**: Dr. Finkelman asked me to see this patient two days after admission and he told me he believed the patient had a brain tumor. The physical findings were indicative of a space occupying lesion and it was felt that his physical condition was such that surgery could be accomplished successfully. It was believed that the only chance the patient had was with surgery. I saw the patient again nine days after surgery because he was running a fever, and the physical findings at this time indicated a left lower pneumonitis. He was treated adequately with antibiotics and responded very well.

That is all I can contribute to this conference, but I have one question I should like to ask our neurologic and neurosurgical colleagues. I should like someone to please tell me what the Mendel-Bechterew reflex is. We are so busy learning eponyms that, even though I teach physical diagnosis including some neurology, this is one I have to learn so I can tell the students.

*Dr. B. W. Lichtenstein***: I had the opportunity of examining the brain after formalin fixation as well as the microscopic preparations. It is evident from Dr. Finkelman's discussion that the patient manifested signs of increased intracranial pressure. Dr. Tinsley removed tissue from the parietal region but it failed to contain tumor (Fig. 2). The microscopic slides of the biopsy specimen consisted of extravasated blood, necrotic tissue and glial nuclei. In one field, there was a rather dense polymorphonuclear leucocytic infiltration. This does not indicate that we are dealing with a brain abscess since necrotic tissue is capable of inducing a polymorphonuclear reaction. Dr. Finkelman described the surgeon's reaction to the biopsy report. All pathologists are agreed that it failed to reveal normal brain and that there was no unequivocal evidence of neoplasm.

Examination of the base of the brain after removal of the hindbrain by a section through the midbrain revealed a

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**Attending, Mount Sinai Hospital.

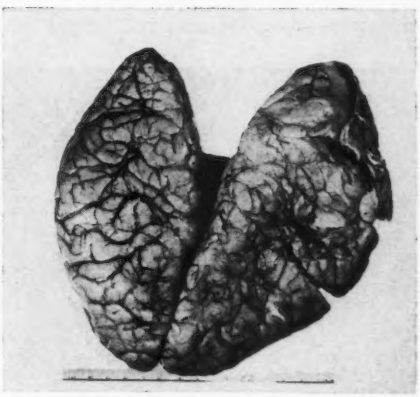
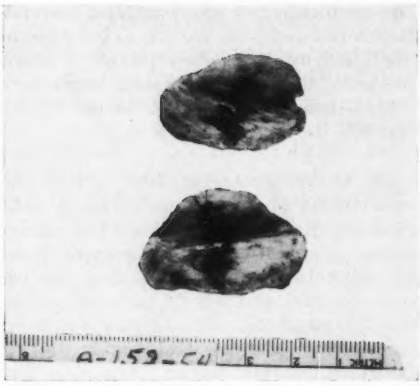


Figure 2. Superior surface of the brain showing the enlargement of the entire left hemisphere and site of the previous biopsy of the parietal lobe.



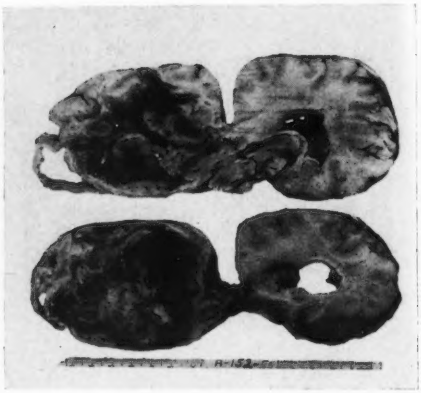
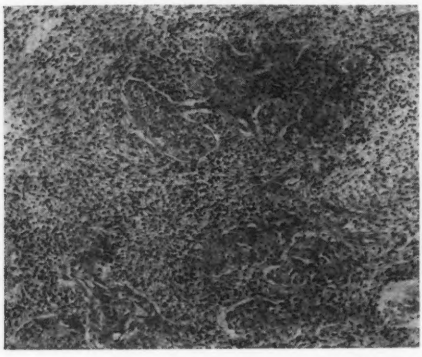


Figure 3 (left). Coronal sections of mid-brain and pons showing extensive hemorrhage.
Figure 4. Coronal sections of the brain showing the tumor involving the major portion of the cross-section. Note that it does not quite reach the biopsy site.

marked swelling of the posterior portion of the left cerebral hemisphere (Figs. 3 and 4). This could be correlated with a right hemiplegia and aphasia in a right-handed individual. The entire left occipital region is replaced by a tumor and there is some extension into the splenium of the corpus callosum. The tumor did not extend to the convex surface and, therefore, could not appear in the biopsy. A microscopic section prepared from a coronal section of the brain (Fig. 5) exhibited the classical features of glioblastoma multiforme. The tumor is large, hemorrhagic, cystic, with many necrotic light yellow and purplish gray areas. The neoplasm extended into the corpus callosum and into the right occipital lobe.

Other sections demonstrate an important clinical-pathological relationship. In a transverse section through the midbrain, one can see multiple focal and confluent hemorrhages throughout the tegmentum. These changes produced the terminal state and death, and not the glioblastoma *per se*. Any condition that produces edema, swelling or increase in size of the cerebral hemispheres is followed by herniation of cerebral tissue through the incisura of the tentorium with resultant compression of the vascular supply of the midbrain. Similar hemorrhages about the aqueduct of Sylvius



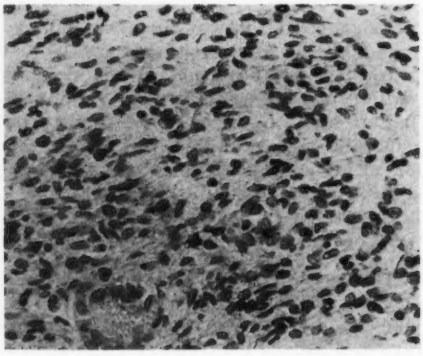


Figure 5 (left). Microphotograph (100X) showing glioblastoma multiforme with predominance of polar spongioblasts. Note the hyperplastic vessels characteristic of glioblastoma multiforme.
Figure 6. Micro-photograph (280X), same as figure 5.

have been observed in cases of cerebral apoplexy and cerebral thrombosis. Loss of consciousness may result from many lesions, the smallest area producing this state being a lesion in the tegmentum of the mesencephalon. Finally, in a high power photomicrograph (Fig. 6), the detailed morphological picture of glioblastoma multiforme can be seen; namely, pleomorphism, many mitotic figures, and large numbers of giant cells.

Dr. I. Finkelman: In answer to Dr. Coe, I usually elicit the Babinski only, or sometimes also the Oppenheim, Gordon or Chaddock signs. There is also the Mendel-Bechterew which consists of a flexion of the small toes on percussion of the dorsal surface of the cuboid bone with a reflex hammer. Babinski reported a simple test which became a classical sign in neurology. Other neurologists have tried to modify this test and there are many of these.

Dr. B. W. Lichtenstein: In closing, I wish to emphasize two points: Dr. Finkelman was correct in stating that the clinical course was that of a neoplasm and, in some cases, such a neoplasm presents a sudden progression and may look like a cerebral vascular accident. If the tumor behaves like a "stroke," it is due to extensive hemorrhage or encephalomalacia and complete removal of such a neoplasm with resultant cure is impossible. In short, the differential diagnosis between vascular accident and neoplasm may be exceedingly difficult from a clinical point of view but the outcome will be more or less the same.

We must also say something in defense of the biopsy. The pathologist can only examine what is removed. It may be very difficult for the surgeon to differentiate the neoplasm from the softened or gliotic tissue about it and, for this reason, absence of proof of a tumor is not proof of its absence. Angiography, pneumo-encephalography, and ventriculography may be used to localize the tumor but our patient was far too ill to be subjected to such procedures. Because of the very evident right hemiplegia and aphasia, Dr. Tinsley decided to operate directly but he could not have known that the neoplasm was in the inferior portion of the left parietal occipital region.

SCHOOL NOTES AND NEWS

FACULTY NEWS

President John J. Sheinin has announced the appointment of Dr. Irving C. Sherman as Professor and Head of the Division of Neurology in the Department of Psychiatry and Neurology. With great pride, the QUARTERLY joins the Faculty and student body in extending a warm welcome to Dr. Sherman.

Born in 1910 in Toronto, Canada, Dr. Sherman attended the University of Toronto and received the degree of Doctor of Medicine in 1933. Dr. Sherman served his internship at Victoria Hospital, London, Ontario, and at Michael Reese Hospital, Chicago, Illinois. He continued his post graduate medical education in the Department of Neurology at Northwestern University Graduate School from 1936 to 1938 and as a Fellow in Psychiatry at Research and Educational Hospital, Chicago, Illinois, from 1939-1941. He was licensed to practice medicine in Illinois in 1938. In 1942 he was certified by the American Board of Psychiatry and Neurology.

In 1936 he was appointed Clinical Assistant in the Department of Neurology and Psychiatry at Northwestern University Medical School. He was promoted to Instructor in 1941, Associate in 1943, and Assistant Professor in 1949.

Dr. Sherman is attending Neurologist at Richael Reese and Cook County Hospitals and Consulting Neuropsychiatrist at Lewis Weiss Memorial Hospital. At Michael Reese Hospital he is in charge of Neurological training of residents in Psychiatry and Medicine.

Despite a very active private practice and extensive activities in the field of medical education, Dr. Sherman has published over thirty scientific papers dealing with problems in the field of Neurology and Psychiatry.

Dr. Sherman is a member of Sigma XI, A.O.A., the American Medical Association, American Psychiatric Association, American Academy of Neurology, Central Neuropsychiatric Association, American League against Epilepsy, Illinois State Medical Society, Illinois Psychiatric Association and Chicago Neurological Society.

In his new position, Dr. Sherman will be in charge of the didactic and bed-side teaching program in Neurology. We feel sure that his association with the Chicago Medical School will be long and mutually beneficial.

* * * *

President John J. Sheinin has also announced the appointment of Dr. Paul B. Szanto as Associate Professor of Pathology.

Born in 1904 in Budapest, Hungary, Dr. Szanto received his medical education at the University of Vienna, and was awarded the degree of Doctor of Medicine in 1929. He served an internship at University Hospital, Tuebingen, Germany in 1929.

Dr. Szanto took his residence training in Pathology at Rudolph Virchow Hospital, Berlin, Germany, in 1930; University Hospital, Tuebingen, German from 1931 to 1932; and Jubileum Hospital, Vienna, Austria, from 1933 to 1934.

In 1938 he arrived in the United States and was licensed to practice medicine in Illinois in 1940. Dr. Szanto served in the United States Army Medical Corps from 1944 to 1946, and held the rank of Captain at the time of his discharge.

Dr. Szanto was certified by the American Board of Clinical Pathology in 1945 and the American Board of Pathologic Anatomy in 1947.

He served as an instructor in Pathology at the Chicago Medical School from 1943-1944. In 1946 Dr. Szanto was appointed to the Department of Pathology of the Northwestern University Medical School where he held the rank of Associate and later Assistant Professor. In 1953 he became Clinical Assistant Professor of Pathology at the University of Illinois College of Medicine and was promoted to the rank of Clinical Associate Professor in 1955.

Dr. Szanto has published nearly forty scientific papers in the field of Pathology and in recent years has concentrated on the diseases of the liver. Since 1946 he has been Associate Director of the Department of Pathology of the Cook County Hospital where many of us have come to know him and have profited by his teaching.

Dr. Szanto is a Fellow of the American Medical Association, the American College of Pathologists, and American Society of Clinical Pathologists, and is a member of many other scientific societies.

We know that Dr. Szanto will be an inspiring teacher in the Pathology courses of the sophomore year and QUARTERLY joins the faculty and student body in welcoming him.

ALUMNI NEWS

Class of 1947

Dr. Jerome A. Ehrlich has returned from the Armed Services and has opened his office for the general practice of medicine at 3 Burbanks Street, Yonkers, New York.

Dr. Frank M. Rampello has been discharged from the United States Army and announces the opening of his office at 363 Graham Avenue, Brooklyn, New York.

Class of 1950

Dr. J. Marvin Wertheim announced the opening of his office for the practice of medicine at 332 Willis Avenue, Roslyn, New York.

Class of 1954

Dr. Eugene Trachtenberg has opened his office for the general practice of medicine in Steltin, New Jersey.

Dr. Herbert L. Snyder announces the opening of his office for the general practice of medicine at 29 Haynes Street, Manchester, Connecticut.

Dr. Bernard B. Boodin has entered general practice at 458 Ford Avenue, Lafayette Estate, Fords, New Jersey.

Dr. Leonard Graivier was married to the former Miss Pauline F. Pierce of Dallas, Texas on July 16th, 1955 and is now awaiting orders from the United States Air Force.

Lietenant (j.g.) Warren Zibelman will be on duty with the North Pacific Fleet from August, 1955 to August, 1957.

First Lieutenant Lionel A. Bernson is at present at the 97th Tactical Hospital, S.A.C., Briggs Air Force Base, Texas.

Dr. Sanford A. Kaplan is awaiting orders from the United States Air Force.

Dr. Allan Berger and the former Miss Gladys Hefter of Chicago were married on June 28th, 1955. He is now stationed at Fort Hood, Texas with the United States Army Medical Corps.

Dr. Eugene Sherman is now an assistant resident in obstetrics and gynecology at University Hospital, Baltimore, Maryland.

Dr. George J. Paul is a resident in surgery at Nassau Hospital, Mineola, New York.

Dr. Howard M. Rosenblatt is a resident in orthopedics at Cook County Hospital.

Dr. Melvin H. Samuels is a resident in pediatrics at Los Angeles County Hospital.

Dr. Hubert B. Segal is a resident in surgery at Brooklyn Jewish Hospital.

Dr. Alfred Faber is a resident in radiology at Crile Veteran's Hospital, Cleveland, Ohio.

Dr. Herbert J. Fisch was married to the former Miss Elinor Medoff of Forest Hills, New York on August 7th, 1955. He is now a resident in anesthesiology at Mount Sinai Hospital, New York.

Dr. Eugene Leibsohn is now a resident in dermatology at Boston City Hospital, Boston, Massachusetts.

Dr. Wallace Bill Katt is an assistant resident in obstetrics and gynecology at Brooklyn Jewish Hospital.

Dr. Marvin F. Levinson was married to the former Miss Janet Liff on July 31st, 1955 and is at present on active duty with the United States Navy.

Dr. Norman S. Lovell is a resident in surgery at Highland Alameda County Hospital, Oakland, California. His second son, Craig, was born on April 27th, 1955.

Lieutenant (j.g.) Lawrence Feuerman is stationed at the United States Naval Hospital, Bainbridge, Maryland.

Lieutenant (j.g.) Samuel P. Shattan is stationed at the United States Marine Corps Air Station Dispensary at Quantico, Virginia.

Dr. Harold J. Sobel was married to the former Miss Geraldine Sobel on July 31st, 1955. He is now a resident in pathology at Maimonides Hospital, Brooklyn, New York.

Dr. Martin E. Weiler is a resident in psychiatry at the New York State Psychiatric Institute, Columbia Medical Center.

Dr. J. C. Rosenberg is in Austria as a Fulbright Fellow in Pathology.

Dr. Sheldon L. Schein is a resident in obstetrics and gynecology at Long Island College Hospital, Brooklyn, New York.

Dr. Jerrald L. Sharoff is a resident in obstetrics and gynecology at Morrisania Hospital, Bronx, New York.

Dr. Saul S. Zimmerman is a resident in anesthesiology at Brooklyn Veterans Administration Hospital.

Dr. Michael West is a resident in internal Medicine at West Side Veterans Administration Hospital, Chicago, Illinois.

First Lieutenant Saul N. Meyer, on active duty with the United States Air Force, is stationed in Scotland.

Lieutenant (j.g.) Melvin Post is stationed at the Portsmouth Naval Base, Portsmouth, New Hampshire.

Dr. Arthur D. Pesin is a resident in pediatrics at Mount Sinai Hospital, New York.

BOOK REVIEWS

CANCER CELLS by E. V. Cowdry. Cloth. First Edition. 677 pages with 137 illustrations. Philadelphia: W. B. Saunders Company, 1955. $16.00.

Dr. Cowdry's book is very different from the several recent publications on current advances in cancer research in that it devotes relatively more space to personal viewpoints and to theoretical concepts than to factual material. Without doubt the best part of the book is contained in Chapters 3 and 4 which deal with the cytology of the tumor cell and compare it with the normal.

It is perhaps a shame that these two chapters have not been separated from the rest and published as a small monograph. Such a volume would undoubtedly have a wide appeal and would meet a need since the cytological descriptions have the great virtue of being considered in the context of the dynamic process of neoplastic growth. The remainder of this volume cannot be recommended except to investigators in the field to whom the mature reflections of the author will be of considerable interest. Insofar as the student and practitioner of medicine are concerned the book is so curiously selective as to be misleading. It is difficult to discern why the author has deemed it necessary to include chapters on the range of human tumors, and on their treatment and diagnosis, and then treat these important subjects in a cursory fashion. A list of human tissues giving rise to tumors is given in alphabetical order; certain tumor types arising from these tissues is then given. None of the commoner neoplasms are mentioned, and we thus have bronchus associated with myoblastoma, skin with mucus secreting tumors and the breast with cystosarcoma phylloides. No particular reason is advanced for this curious arrangement. In the experimental sections concerning carcinogenesis with chemicals and viruses only a few selected facts are presented; the major part of the text is devoted to the presentation and discussion of theories.

The volume provides the reader with an education in philology—almost every medical or biological term used having its derivation explained, and also with a useful list of addresses of important cancer research workers and laboratories.

P. Shubik, Director,
Division of Oncology.

* * * * *

BASIC SURGICAL SKILLS by Robert Tauber, M.D. Cloth. First Edition. 75 pages, illustrated. Philadelphia: W. B. Saunders Company, 1955. $3.75.

It is remarkable to think that the do-it-yourself influence should spread to the field of surgery, and this book is an indication that it has. It endeavors to teach the basic skills of knot tying and suture handling away from the operating room. First, a training board is described for the do-it-yourselfer to construct; it is a contrivance of wood, rings, hooks, and gauze sponges. Knots, sutures, and methods of hemostasis are then described as exercises to be performed on the board.

Actually, the title is a misnomer; Dr. Tauber is a gynecologist and the techniques described are those which have found favor in gynecological surgery. Some are frowned upon in general surgery. Many of the techniques most commonly used in general surgery are not even mentioned. Some of Dr. Tauber's special interests, such as his twin stitch and stump stitch, are described.

Most students and residents practice techniques on sponges, belt loops, cloth, and other things whose number is limited only by their imaginations. It is a good thing for a book with a series of exercises for practice of surgical techniques outside of the operating room to be published. The loose leaf type of binding is an asset. The illustrations are line drawings and are adequate, but not exceptional. The exercises, however, are not a well rounded series for the needs of general surgery.

* * * * *

CARDIAC DIAGNOSIS — A PHYSIOLOGIC APPROACH by Robert F. Rushmer, M.D. Cloth. First Edition. 447 pages. Illustrated. Philadelphia: W. B. Saunders Company, 1955. $11.50.

This volume by Dr. Rushmer is a well written presentation of basic cardiovascular physiology and its value in the diagnosis of cardiac disease. The book is divided into five parts.

The first two parts deal with the function of the normal cardiovascular system and its regulation. These sections will give those far removed from their basic science courses an excellent review of the more basic principles necessary for the understanding of the pathophysiology and symptomatology of this organ system.

The third section is concerned with congestive heart failure and the discussion, here, is brief when compared to some of the more encyclopedic volumes on cardiac disease.

Part four is devoted to the methods and techniques used in cardiac diagnosis. For the purposes of the book, this section is adequate, but some previous knowledge on the part of the reader must be presumed. For instance, the chapter on electrocardiography is compact and moves far too quickly for the uninitiated in the field.

The final section is concerned with diagnosis and the topics of myocardial ischemia, myocarditis, valvular disease, and congenital heart disease are discussed. The subjects covered in the previous sections are integrated and correlated for the objective of accurate diagnosis.

For the purposes of review and/or gaining a new point of view on the subject of cardiac diagnosis, this book should be of value to the senior medical student, pediatricians, internists, and general practitioners.

CLINICAL PATHOLOGIC CONFERENCE OF COOK COUNTY HOSPITAL. Volume I. Cardiovascular-Renal Problems. Edited by Hans Popper, M.D., Ph.D. and Daniel S. Kushner, M.D. First Edition. 325 pages. New York: The Blakiston Company, Inc., 1954. $5.00.

Many physicians who have taken part of their training at Cook County Hospital remember the very wonderful clinico-pathologic conferences held each week in the morgue amphitheater. Some of us have yearned for a more permanent record of the discussions which transpired. Doctors Popper and Kushner have met this desire with the first volume of a contemplated five volume series which will publish conferences held during 1946 to 1953 dealing with problems of gastroenterology, hematology, metabolic, and neoplastic disease.

The first volume deals with twenty-six problems of differential diagnosis in Cardiovascular-renal disease. For the convenience of the reader, the book is indexed according to both the clinical problem and the pathologic diagnosis. Each case is presented under the following headings: clinical data, clinical discussion, pathologic observations (by Dr. Popper), clinical pathologic correlation, and final pathologic diagnosis. The cases selected are informative and make for interesting reading. They are adequately illustrated in black and white photographs showing gross and microscopic pathology. The book will be a worthy addition to any clinician's or student's library.

* * * * *

HENRY FORD HOSPITAL INTERNATIONAL SYMPOSIUM ON CARDIOVASCULAR SURGERY. Edited by Conrad R. Lam, M.D. Cloth. First Edition. 543 pages. Illustrated. Philadelphia: W. B. Saunders Company, 1955. $12.75.

This new volume is the transcription of a series of papers presented at an international symposium on Cardiovascular Surgery: Recent Studies in Physiology, Diagnosis and Techiques held at the Henry Ford Hospital in March, 1955. The volume, as edited by Dr. Lam, covers a wide range of topics all of which are of interest to the student of cardiovascular surgery. It includes both original papers and panel discussions on such problems as diagnostic techniques, pulmonary stenosis, adjustments between the systemic and pulmonary circulation, evaluations of transposition operations, mitral stenosis and mitral insufficieny, aortic stenosis and aortic insufficiency, septal defects, cardiac arrest, hypothermia, and aneurysms and occl,usive diseases of the aorta. The book is clearly written, brief, and adequately illustrated.

* * * * *

TEXTBOOK OF ENDOCRINOLOGY. Edited by Robert H. Williams, M.D. Cloth. Second Edition. 776 pages with 175 illustrations. Philadeplhia: W. B. Saunders Company, 1955. $13.00.

In a rapidly developing field such as endocrinology, this text does an admirable job of keeping pace with current progress. The volume is divided into chapters on the various endocrine glands, and each begins with a discussion on anatomy, physiology, and physiopathology which progresses from the most elementary concepts to a thorough description of the endocrinopathies. Diagnosis is carefully discussed, and treatment presented in sufficient detail to enable management of a case. Secondary manifestations and complications are also discussed in some detail. In addition, there are sections on general principles of the physiology of the endocrines, influence of the endocrine glands on growth and development, neuroendocrinology, obesity, laboratory diagnostic and assay procedures, and hormone preparations. Experimental work is well integrated, and a fair number of charts and illustrations are present. On controversial topics, the evidence and most prominent theories are described with objectivity. The book is admirably suitable as a text and reference for students and the non-specialist, being concise, reliable, and unusually readable.

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